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Observations upon Metabolic changes during  
and after Exercise.

submitted for the degree of  
Master of Science  
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by

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## Introduction

Gerhardt in 1865 discovered acetoacetate in the urine of diabetic patients, but a further 80 years passed before coenzyme A was discovered, linking the intermediary metabolism of fat and ketone bodies. Disturbances of carbohydrate metabolism accompanied by ketosis also occur during fasting and when the diet contains large quantities of fat, and in addition there are several other physiological conditions, involving some sort of stress to the body, in which ketone bodies accumulate. One such condition is prolonged exercise, long known to be associated with alterations in fat metabolism.

In 1936 Courtice and Douglas reported an increase in ketone bodies in their blood and urine after walking for 10 miles at 4.5 mph. Drury, Wick and MacKay (1941) found that walking was insufficient to produce ketosis and more intensive exercise (10 mph for 20 min) was required. Post-exercise ketosis has been studied more recently by Passmore and Johnson (1958) who concluded that exercise is ketogenic, in addition to producing a negative calorie balance. Little other work has been done on post-exercise ketosis, possibly because of the protracted nature of the investigations.

In 1953 Lynen and Ochoa discovered that the normal intermediates of fatty acid degradation are the coenzyme A derivatives of ketone bodies, rather than the free ketone bodies. In the same year, Lehninger and Greville showed that the intermediate formed from long-chain fatty acids is L(+)  $\beta$ -hydroxybutyrate coenzyme A, whereas the free  $\beta$ -hydroxybutyrate appearing in body fluids has the D-configuration. Ketone bodies, then, are not normal intermediates in the  $\beta$ -oxidation of fatty acids, and their role in the body only became clearly established recently when it was shown that ketone bodies can serve effectively as fuels of respiration in animal tissues (Williamson and Krebs, 1961; Bergman, Kon and Katz, 1963).

This knowledge of the probable physiological role of ketone bodies, coupled with specific enzymic methods for their determination, has made it possible to look more closely at the metabolism of ketone bodies during and after exercise. A possible approach to this subject was prompted by the observations made on a group of hill walkers, who were fit but not in athletic training. These showed that, after a period of 3-5 hours of walking, most of the subjects had ketonuria on qualitative testing (Cooper, Johnson and Pugh, unpublished observations). In a later

investigation upon runners after they had competed in a marathon race (42 km), the winner of which took 2 hr 38 min, it was found that none of the competitors had ketonuria, even though the exercise was more strenuous (Pugh, Corbett and Johnson, 1967), and Astrand et al., (1963) have also failed to find ketonuria in competitors after an 85 km championship ski race. These results suggested that the phenomenon of post-exercise ketosis might be different in athletes compared with non-athletes.

The observations presented in the first part of this thesis demonstrate that there is indeed a difference between athletes and untrained subjects in the development of post-exercise ketosis. This difference is shown to be related to the individual's fitness and is a result of training. In the second part, the effect upon ketosis of the administration of glucose and acetoacetate is described, and a difference between athletes and untrained subjects in their capacity to metabolise ingested acetoacetate after exercise reported. In the final section, the secretion of growth hormone in response to exercise in each group is described, and observations on the metabolic changes produced by exercise in a group of patients with hypopituitarism included.



The methods used for the analysis of metabolites are described briefly in Chapter 1, but the procedures adopted in each series of investigations are described in each chapter. The thesis concludes with a list of original publications already produced on this research

## CHAPTER 1

### Techniques

This chapter considers the biochemical techniques that have been used in all the experiments. The subjects who took part in each series of investigations will be described in the appropriate chapters along with the different procedure used.

### Biochemical Techniques

- (a) Preparation of blood specimens. 15 ml blood was taken at each sample from an antecubital vein. 10 ml of this was placed in a heparin tube and centrifuged for 10 min at 2500 rpm. The separated plasma was pipetted off and stored at  $-10^{\circ}\text{C}$ . The other 5 ml blood were immediately added to 5 ml 10% ( $^{\text{w}}/\text{v}$ ) ice cold perchloric acid in a universal container. This was shaken vigorously and stored at  $0^{\circ}\text{C}$  until the precipitate could be centrifuged off.

(b) Treatment of denatured blood. The protein precipitate was removed, usually within 24 hr, by centrifugation at 18,000 rpm for 15 min. The filtrate was spun again at 2500 rpm for a further 10 min, decanted into a graduated tube and the volume noted. The centrifugations were carried out at 0°C. The specimens were neutralised with 20% potassium hydroxide using universal indicator and the volume read again after the potassium perchlorate precipitate had been spun down. 1.8-2.0 ml alkali were required. The filtrate was then decanted into plastic tubes which were stored at -10°C for subsequent analysis. A dilution factor was calculated from the volume of blood taken and the volume of alkali added. Spectrophotometric measurements were carried out on either a Zeiss PMQ 11 or a Uni SP 500 Mk 11.

(c) Analysis of blood pyruvate and blood acetoacetate. These two metabolites were measured on the same sample by modifications of the methods of Bucher et al. (1963), and Williamson, Mellanby and Krebs (1962).

The pyruvate was reduced to lactate with excess reduced nicotinamide adenine nucleotide (NADH) using a phosphate buffer (0.1M, pH 7.0). The reaction was catalysed by lactic dehydrogenase and the decrease in optical density due to the oxidation of NADH measured at 340 m $\mu$ . The acetoacetate was assayed under the same conditions by adding the enzyme D-(-)- $\beta$ -hydroxybutyric dehydrogenase, which catalyzed the reduction of acetoacetate to D-(-)- $\beta$ -hydroxybutyrate. Acetoacetate is slowly decarboxylated in neutral solution so this assay had to be carried out immediately after the samples were neutralised. The acetoacetate remained stable in acid solution (i.e. after the samples had been deproteinised) for up to 1 week.

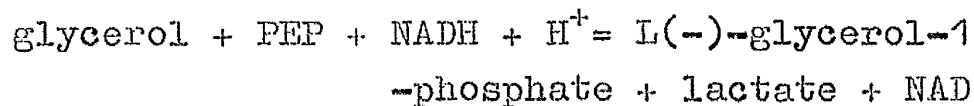
- (d) Analysis of blood glucose. Blood glucose was determined by the method of Bergmeyer and Bernt (1963) using glucose oxidase and peroxidase. Glucose oxidase catalyses the conversion of D-glucose to D-gluconic acid and the hydrogen peroxide also formed is decomposed by peroxidase, the oxygen liberated oxidising the colourless o-dianisidine to a coloured derivative.

The optical density of the reaction mixture was measured at 440 m $\mu$  and related to glucose standards of different concentrations.

Duplicate samples of 0.05 ml were analysed and the reaction mixture was incubated at 37°C for 1

- (e) Analysis of blood  $\beta$ -hydroxybutyrate. This was determined by the method of Williamson, Mellanby and Krebs (1962) using the enzyme D- $\beta$ -hydroxybutyric dehydrogenase to catalyse the oxidation of D-(-)- $\beta$ -hydroxybutyrate to acetoacetate in the presence of excess nicotinamide adenine dinucleotide (NAD). An alkaline medium was used (pH 8.5) and the acetoacetate removed in the form of its hydrazone, the change in optical density at 340 m $\mu$  due to the formation of NADH being measured.

- (f) Analysis of blood glycerol. This was determined by the method of Kreutz (1962). Glycerokinase was used to catalyse the phosphorylation of glycerol by adenosine 5-triphosphoric acid (ATP) to L-glycerol-1-phosphate. The adenosine 5-diphosphoric acid (ADP) was phosphorylated back to ATP with phosphoenolpyruvate (PEP) forming pyruvate which was then reduced to lactate with NADH. The overall reaction was



The optical density change due to the formation of NAD was measured at 340 m $\mu$ .

- (g) The analysis of plasma nonesterified (free) fatty acids. Plasma free fatty acids (FFA) were assayed by the method of Itaya and Ui (1961). The FFA were extracted from plasma by shaking with chloroform neutralised (pH 7.0) by phosphate buffer (0.1 M). The extracted FFA were complexed with copper-triethanolamine solution and estimated calorimetrically with the dye sodium diethyldithiocarbamate. After shaking the chloroform layer with copper-triethanolamine the water was removed by filtering through ordinary filter paper. Silicon-treated phase-separating filter paper gave falsely high concentrations of FFA. The colour developed by the dye was measured immediately at 440 m $\mu$ . All FFA determinations were carried out in duplicate using 0.2 ml samples of plasma.

## Materials

The enzymes used in the analysis of blood specimens were obtained from Boehringer Corporation, Ltd., Bilton House, London, W.5, except for glucose oxidase (Type 11) which was obtained from Sigma London Chemical Co. Ltd. Other chemicals were obtained from British Drug House, Ltd., Poole, England.

## CHAPTER 2

### Blood Ketone Levels of Healthy Men at Rest

The formation and utilisation of ketone bodies is normally adjusted to prevent any marked accumulation in blood and tissue concentrations. The range of ketone bodies found in healthy persons who are not under any form of stress is small when it is considered that there is no other body constituent which shows concentration changes in a dimension comparable to that of ketone bodies (Wieland, 1968). There are few estimations of normal ketone body levels in large numbers of subjects in the literature, and the majority have depended on chemical methods of analysis which are not always satisfactory and often rely on the determination of the acetone and acetoacetate fraction by 'difference' rather than directly. The development of specific enzymatic methods for the determination of acetoacetate and 3-hydroxybutyrate separately has enabled total blood ketones to be estimated more accurately.

#### Methods.

Venous blood acetoacetate and 3-hydroxybutyrate concentrations have been measured in 83 healthy male subjects (age 18-35). 15 of these subjects were athletes of University standard. All the subjects had fasted for at least 4 hr and were in a resting state.



## Results

Mean resting levels and standard deviations of acetoacetate and 3-hydroxybutyrate in all the subjects were  $0.029 \pm 0.017 \mu\text{mole/ml}$ , and  $0.053 \pm 0.045 \mu\text{mole/ml}$ , giving a mean total ketone body concentration of  $0.077 \pm 0.056 \mu\text{mole/ml}$ , and a mean hydroxybutyrate/acetoacetate ratio of  $2.10 \pm 1.03$ .

There was no significant difference between total ketone bodies in the athletes ( $0.062 \pm 0.043 \mu\text{mole/ml}$ ), and in the other group ( $0.081 \pm 0.046 \mu\text{mole/ml}$ ).

22 subjects who had fasted for 12 or more hours had a mean of  $0.079 \pm 0.059 \mu\text{mole/ml}$ , and this was not significantly different from the mean level found in 45 subjects who had fasted for between 4 hr and 8 hr ( $0.078 \pm 0.055 \mu\text{mole/ml}$ ).

## Discussion

Similar values have been obtained for non-diabetic subjects (mean  $0.107 \mu\text{mole/ml}$ ) by Werk and Knowles (196) who used a chemical method of analysis developed by Greenberg and Lester (1944). Johnson, Sargent and Passmore (1958) reported a mean value of  $0.71 \mu\text{mole/ml}$  for total serum ketones in 208 healthy young men fasted overnight. It has been reported (Stark and Somogyi, 19) that the concentration of total ketone bodies in the plasma is approximately twice that in the red blood

1

cells. If this is so, the corresponding figures for whole blood for the observations of Johnson and his associates would still be considerably higher than those reported here. Other workers who have obtained similar values to the present results include Lyon and Bloom (1958), and Stark and Somogyi (1943).

The ratio of 3-hydroxybutyrate to acetoacetate has been reported as 2 (Stark and Somogyi, 1943), 1.78-4.3 (Williamson, Krebs and Mellanby, 1962) and ranging from 1-20 (Mayes and Robson, 1957). Peden (1964), describing normal values in infants, gives a ratio of 0.5-0.7.

Werk and Knowles (1961) describe a different value of ketone bodies in healthy men after fasting  $3\frac{1}{2}$  hr and 14 hr, but the present observations do not indicate any significant difference between fasting of 4-8 hr and fasting of 12 hr.

### Summary

The mean level and standard deviation of total blood ketone bodies (acetoacetate plus 3-hydroxybutyrate) in 83 healthy male subjects in a resting state was  $0.077 \pm 0.056 \mu\text{mole/ml}$ . There was no difference in resting levels between athletes and untrained subjects nor between subjects who had fasted for 4-8 hr and those who had fasted more than 12 hr.

Changes in Blood Metabolites during and after Exercise

This chapter describes the effect of severe exercise on the levels of blood metabolites, with particular reference to differences between athletes and healthy untrained subjects. Blood samples were taken during the exercise period because different lactate and pyruvate responses to exercise have been previously related to physical fitness, and also for some time after the exercise to follow the development of post-exercise ketosis. The references quoted in the introduction indicated that this might also be related to athletic ability, and in the first part of this chapter clear differences in the blood levels of ketone bodies and their metabolic precursors, FFA, are described in athletes and untrained subjects. In the second part of this chapter a shorter period of moderate exercise with the subjects exercising at similar work rates on an ergometer is also found to be accompanied by changes in metabolic concentrations which differ in degree in trained and untrained subjects.

## Differences between Athletes and Non-Athletic Subjects during and after running

In this section the differences in the concentrations of various metabolites have been measured in athletes and untrained subjects before, during and after a period of strenuous exercise.

### Methods

#### Subjects

Nine subjects (aged 22-34 yr) trained regularly by running 60-150 km/week, in order to compete in middle and long distance events (15-42 km). Their mean weight was  $64.3 \pm 3.5$  kg (S.D.), and mean height  $174.5 \pm 6.1$  cm (S.D.). The 18 untrained subjects (aged 20-23 yr) were University students who were not regularly taking part in any athletic sport and so were not in training. Their mean weight was  $68.1 \pm 8.0$  kg (S.D.), and mean height  $176.0 \pm 8.3$  cm (S.D.).

#### Procedure

The investigations were carried out between 11.00 - 20.00 hr, and all were started at least 3 hr after the previous meal. Conditions were mild ( $17-22^{\circ}$  C) and dry with little or no wind.

The subjects ran for  $1\frac{1}{2}$  hr on an outdoor track covering a measured distance. Lap times were recorded

and observations then continued for a further 2 hr. The subjects ran at their own speed, often in pairs. They were weighed before and after exercise, and radial pulse rates were measured before running and at 30 min intervals during and after exercise. Blood samples (15 ml) were taken by venepuncture before running, 30 min later, at the end of exercise, and at subsequent 30 min intervals. In one series of investigations in which six subjects (three athletes and three untrained subjects) ran for 2 hr, blood samples were taken at 30 min intervals throughout exercise. In this investigation and later in another series, gut temperatures were measured as an indication of central temperature change, using radio pills (Fox, Goldsmith and Wolff, 1962), made by the Nippon Electric Co., Japan. The pills were swallowed  $\frac{1}{2}$ - $1\frac{1}{2}$  hr before exercise, and observations made at 30 min intervals. Blood samples were analysed for lactate, pyruvate, acetoacetate, 3-hydroxybutyrate, glucose and glycerol, and plasma samples for free fatty acids. The plasma was also analysed for urea, sodium, potassium, chloride, and bicarbonate by means of an autoanalyser.

The results in the next section are expressed as means and standard deviations, and refer to the 27 subjects who ran for  $1\frac{1}{2}$  hr.

## Results

The athletes ran much faster ( $16 \pm 2$  km/hr) than the untrained group ( $10 \pm 3$  km/hr), and they ran at a steady speed, whereas the others showed considerable variation and slowed down progressively (Fig 3; 1). The athletes lost more weight ( $3.29 \pm 0.36\%$  total body weight) than the non-athletes ( $1.8 \pm 0.43\%$  total body weight). The mean heart rates of the athletes were lower than those of the untrained subjects. After  $\frac{1}{2}$  hr and 1 hr of exercise, the athletes and non-athletes had mean heart rates of 126 and 130, and 160 and 165 beats/min respectively. Half an hour after exercise, the mean heart rates were 78 and 102 beats/min respectively (Fig 3:2). In the investigations in which central temperatures were measured both groups had similar initial temperatures, but those of the athletes remained lower during the first hour of exercise in spite of their higher running speed (Fig 3:3).

### Concentration of Metabolites in Blood

#### During and after exercise

##### Lactate

Resting blood lactate levels were similar in both groups of subjects (athletes  $1.31 \pm 0.53$   $\mu$ mole/ml non-athletes  $1.14 \pm 0.41$   $\mu$ mole/ml). The experiment in which blood samples were taken at 30 min intervals

MEAN RUNNING SPEED per  $\frac{1}{2}$  HOUR PERIOD

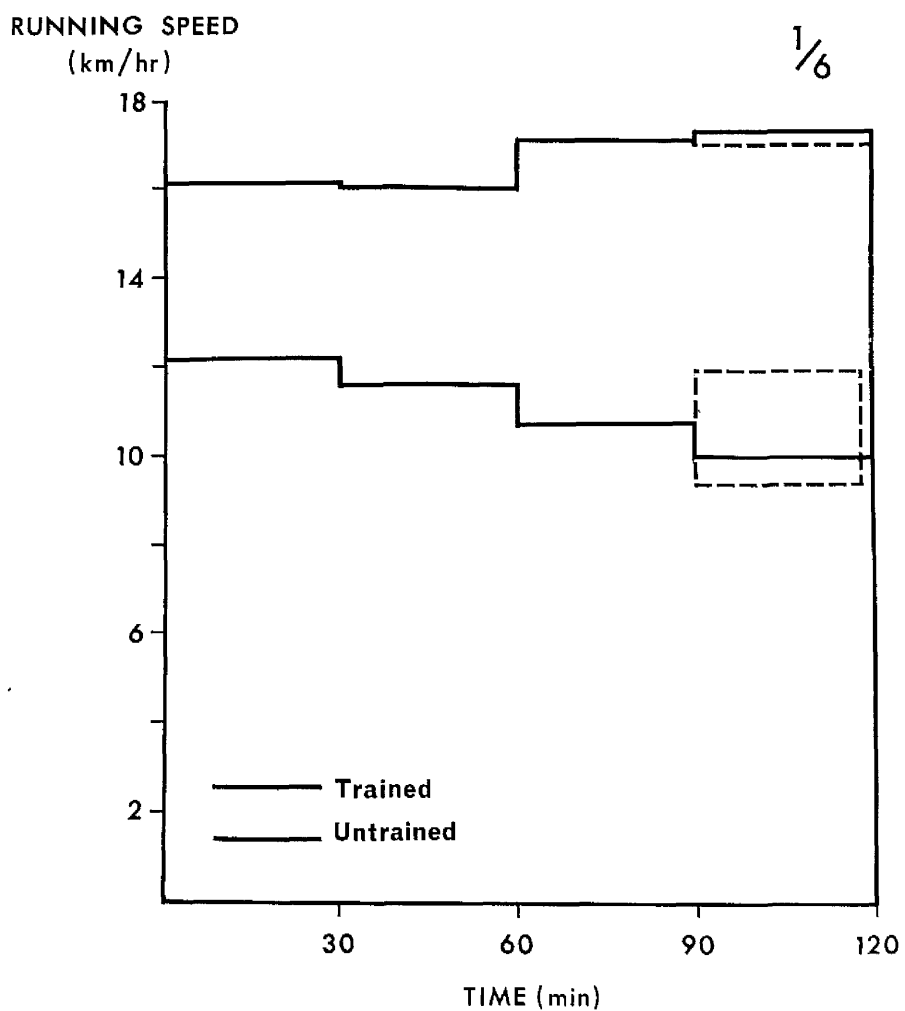


Fig. 3:1 Mean running speed in the three trained athletes (upper line ) and three untrained subjects (lower line ) while running for 2

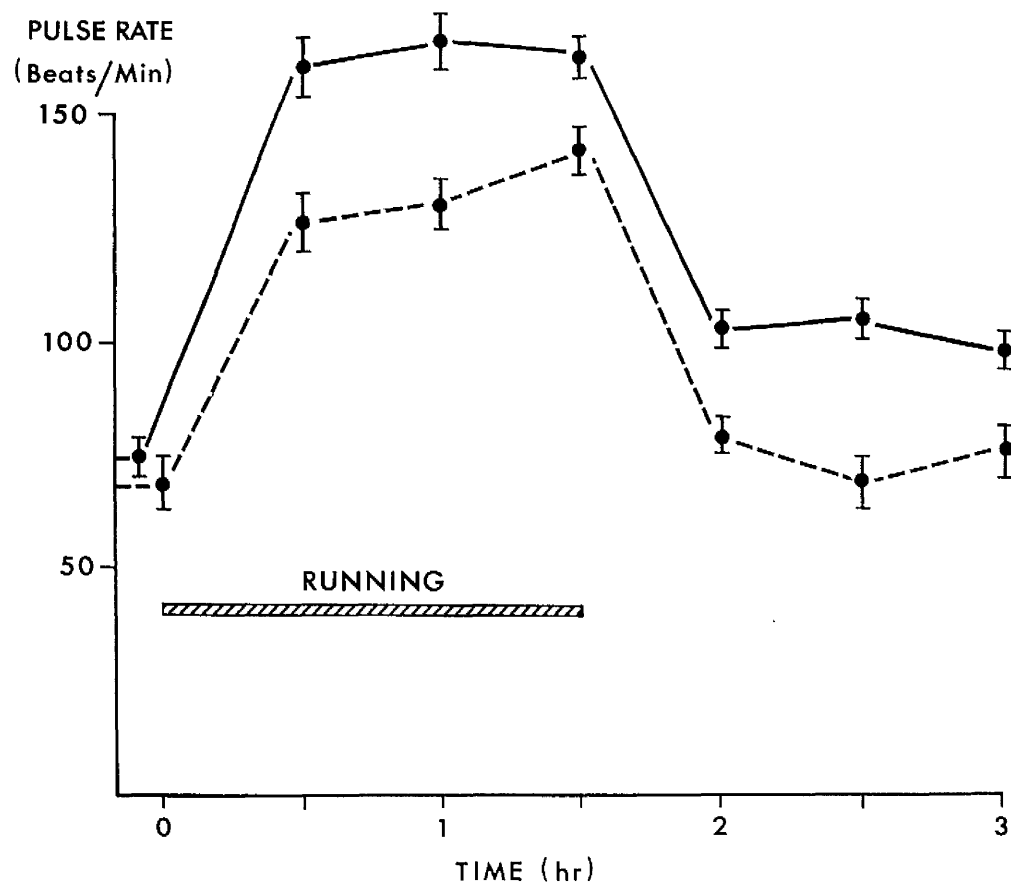


Fig. 3:2 Pulse rates during and after running  $1\frac{1}{2}$  hr in 9 athletes (  $\bullet - - - \bullet$  ) and 18 untrained subjects (  $\bullet - - - \bullet$  ), ( mean  $\pm$  1 S.E.M. ).



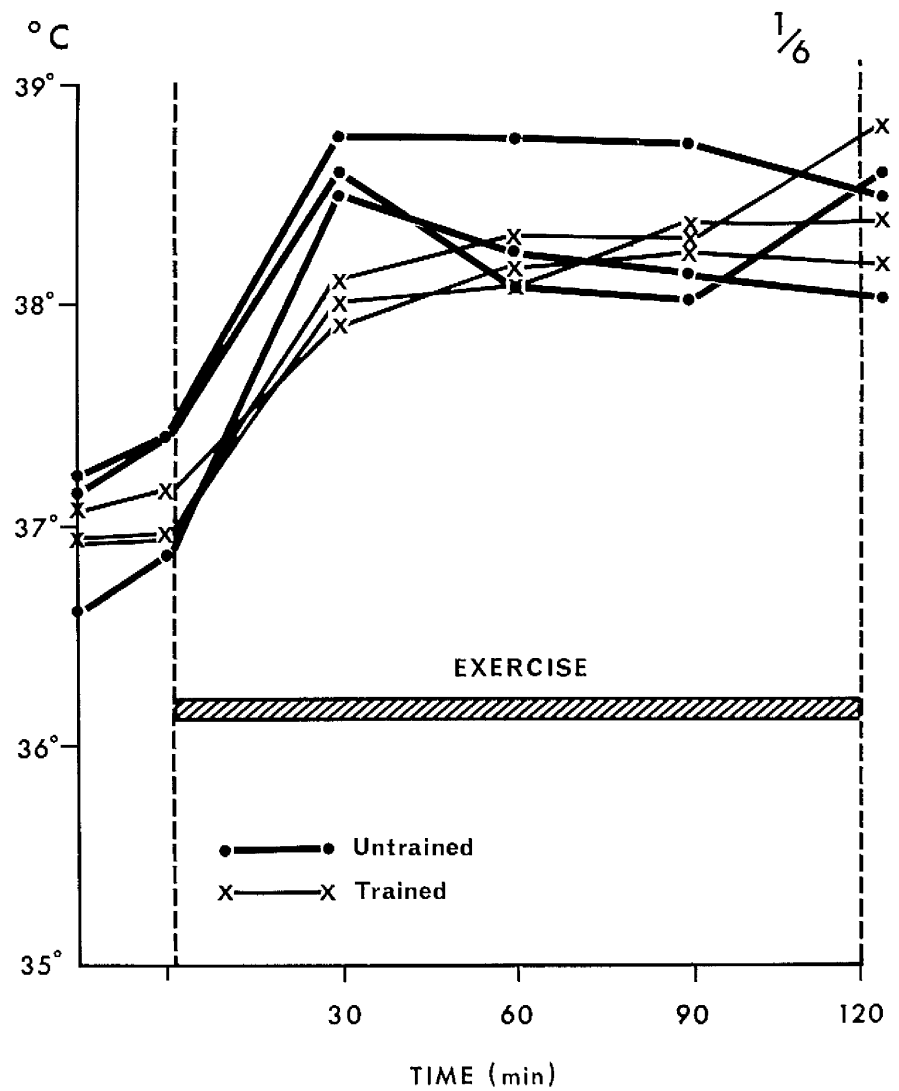


Fig. 3:3 Gut temperatures during exercise in 3 trained athletes ( x—x ) and 3 untrained subjects ( •—• ).

throughout 2 hr of exercise indicated that the greatest difference between the two groups occurred during the early part of exercise and appeared in the 30 min sample, Table 3:1.

At 30 min the athletes had a mean level of  $1.49 \pm 0.62 \mu\text{mole/ml}$ , whereas in the non-athletes the lactate concentrations rose to  $4.63 \pm 2.05 \mu\text{mole/ml}$  after 30 min, and then decreased throughout the rest of the experiment (Fig 3:4).

### Pyruvate

The changes in pyruvate resembled those of lactate (Fig 3:5). Resting levels were similar in both groups (athletes  $0.079 \pm 0.029 \mu\text{mole/ml}$  ; non-athletes  $0.081 \pm 0.023 \mu\text{mole/ml}$ ). The athletes again showed a lower peak after 30 min exercise ( $0.099 \pm 0.035 \mu\text{mole/ml}$ ) compared to the other group ( $0.234 \pm 0.087 \mu\text{mole/ml}$ ).

### Glucose

This showed less marked changes in concentrations (Fig 3:6): resting values were lower in the athletes ( $71.5 \pm 18 \text{ mg\%}$ ) compared to the non-athletes ( $93.8 \pm 11.5 \text{ mg\%}$ ) and after 30 min of running the athletes showed a slight rise ( $86.1 \pm 21.1 \text{ mg\%}$ ), whereas the untrained group had the same or slightly lower value ( $92.0 \pm 14.7 \text{ mg \%}$ ).

Time (min)		Rest	30	60	90	120	150	1
Athletes	1	-	0.78	0.69	1.24	1.61	0.90	
	2	0.99	1.19	1.01	1.47	1.55	1.23	1.
	3	1.45	2.24	1.83	3.57	3.26	1.53	0.
Mean		1.22	1.40	1.18	2.09	2.14	1.22	1.
Non-athletes	1	1.41	7.75	6.17	4.32	2.86	-	2.
	2	1.11	6.50	3.44	3.11	2.78	3.59	1.
	3	1.86	7.38	3.98	2.14	3.92	1.85	1.
Mean		1.46	7.21	4.53	3.19	3.19	2.72	1.

Table 3:1 Blood Lactate Concentrations ( $\mu$ mole/ml) in  
3 Athletes and 3 Non-Athletes Running from 0-120 min

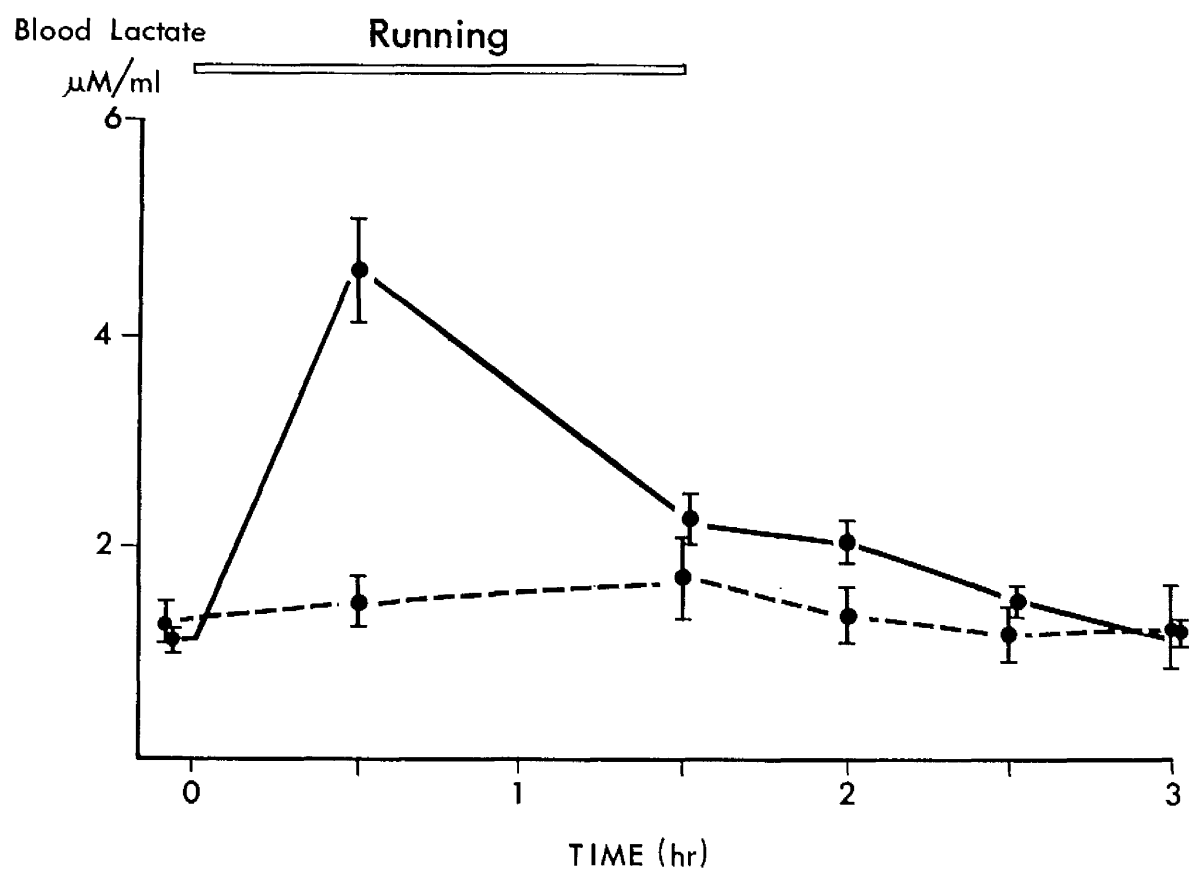


Fig. 3:4 Blood lactate ( $\mu\text{mole}/\text{ml}$ ) in 9 athletes  
 (• - - - •) and 18 untrained subjects (• — — •)  
 (mean  $\pm$  1S.E.M.).

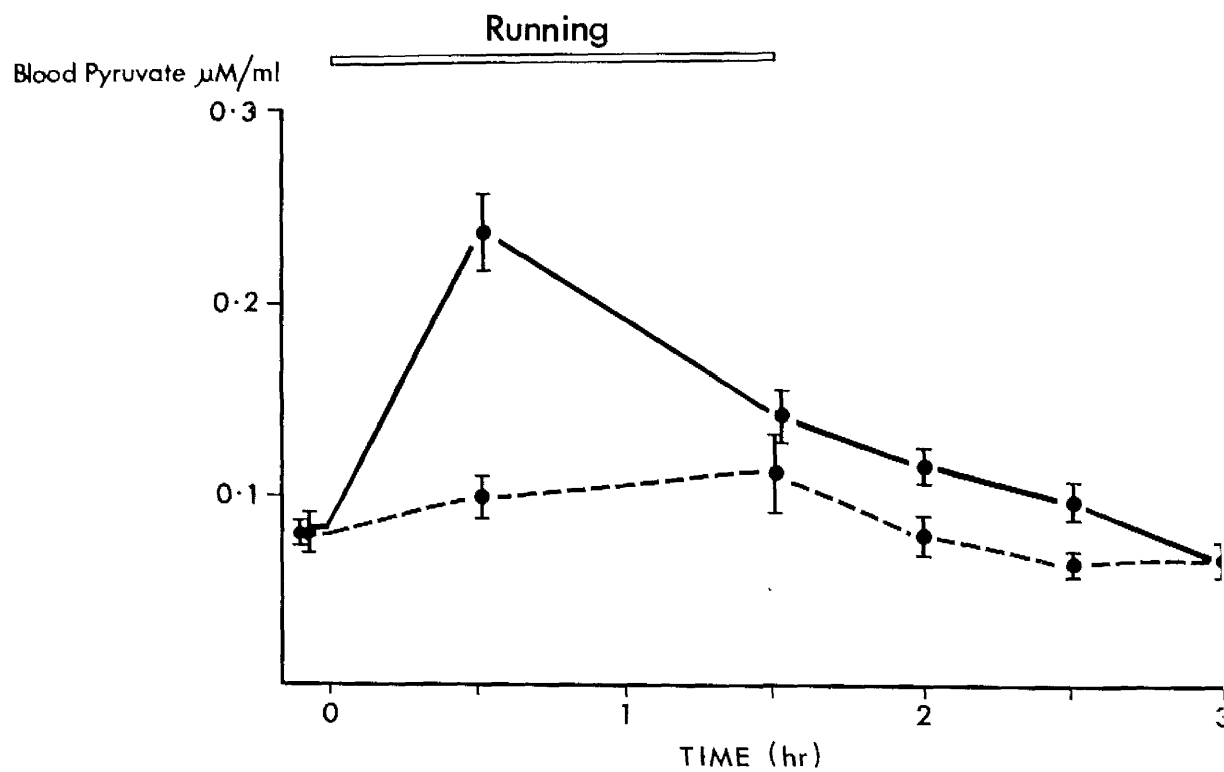


Fig. 3:5 Blood pyruvate ( $\mu\text{mole/ml}$ ) in 9 athletes  
 (---) and 18 untrained subjects (—)  
 (mean  $\pm$  1 S.E.M.).

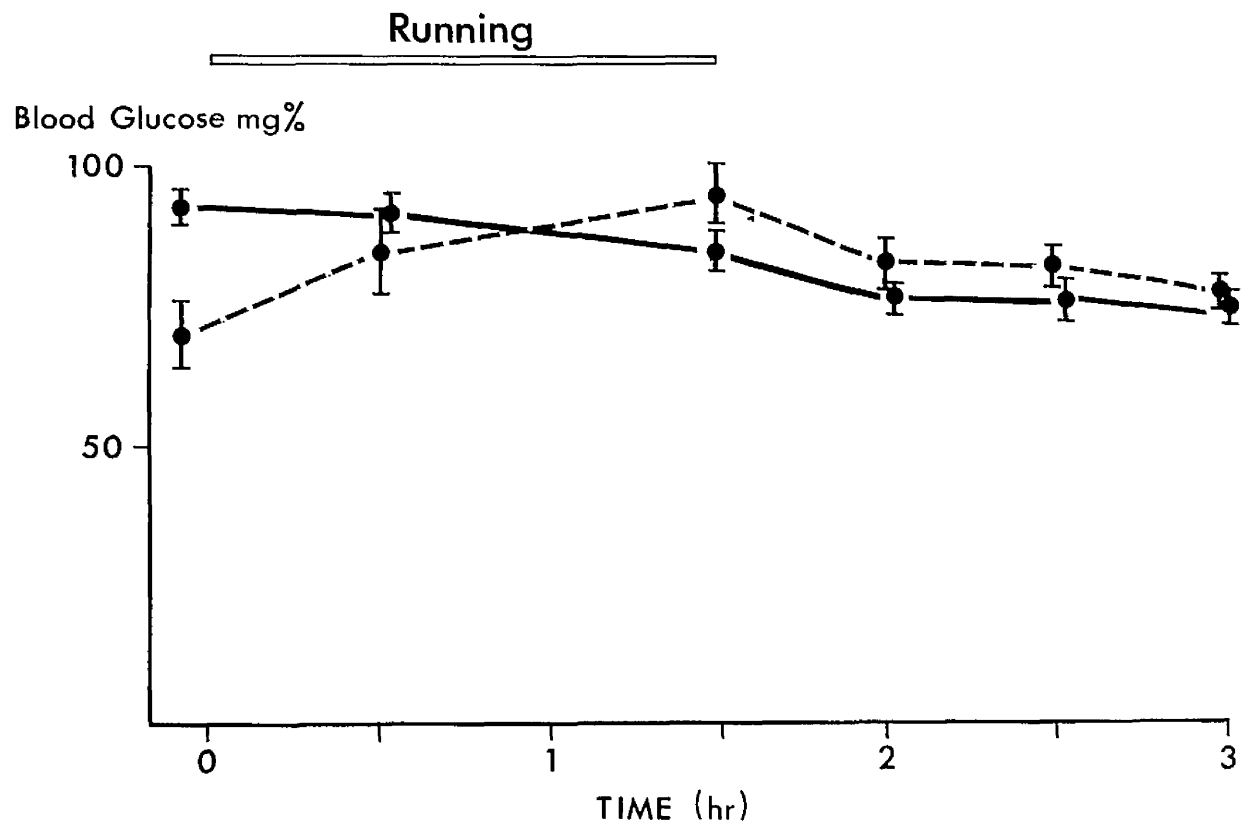


Fig. 3:6 Blood glucose (mg.) in 9 athletes (● --- ●) and 18 untrained subjects (● ——— ●), (mean  $\pm$  1 S.E.M.).

### Glycerol

Blood levels were slightly lower in the athletes at rest ( $0.051 \pm 0.016 \mu\text{mole/ml}$ ) than in the non-athletes ( $0.072 \pm 0.022 \mu\text{mole/ml}$ ) and in both groups the concentration rose to a peak in the sample immediately after the exercise, reaching similar values. After exercise the levels declined (Fig 3:7).

### Free Fatty Acids

The plasma free fatty acids at rest were lower in the trained subjects ( $0.36 \pm 0.13 \mu\text{equiv/ml}$  and  $0.53 \pm 0.33 \mu\text{equiv/ml}$ ) but this was not significant ( $p \gg 0.05$ ). The levels of FFA rose gradually until 1 hr after the end of running, but in the untrained group the levels reached were much higher (Fig 3:8).

### Ketone Bodies

Blood-ketone-body concentrations (acetoacetate plus 3-hydroxybutyrate) also rose during and after exercise. The athletes, however, showed only a slight rise ( $0.18 \mu\text{mole/ml}$ ) in ketone bodies throughout the experimental period, while in untrained subjects they rose markedly ( $0.66 \mu\text{mole/ml}$ ) in the post-exercise period (Fig 3:9). The mean 3-hydroxybutyrate/acetoacetate ratios were similar

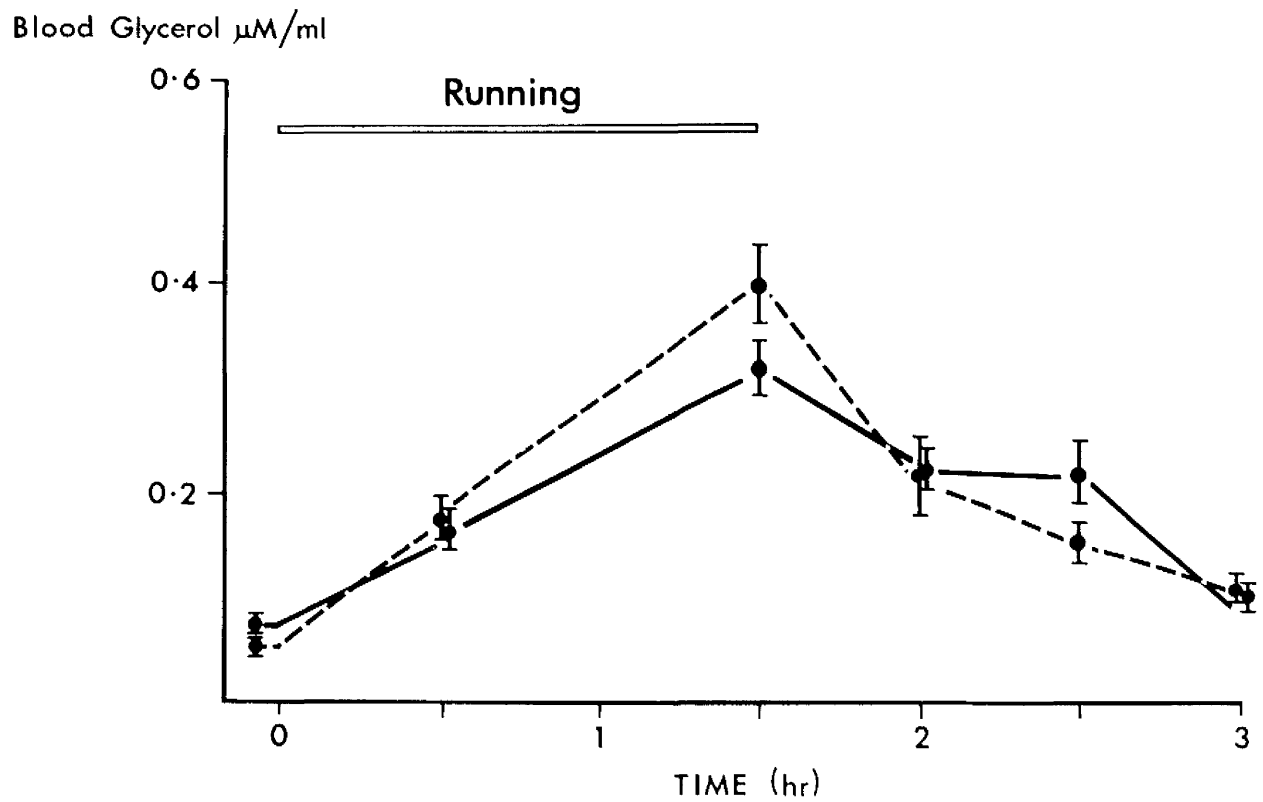


Fig. 3:7 Blood glycerol ( $\mu\text{mole}/\text{ml}$ ) in 9 athletes  
 (---) and 13 untrained subjects (—)  
 ( mean  $\pm$  1 S.E.M.).



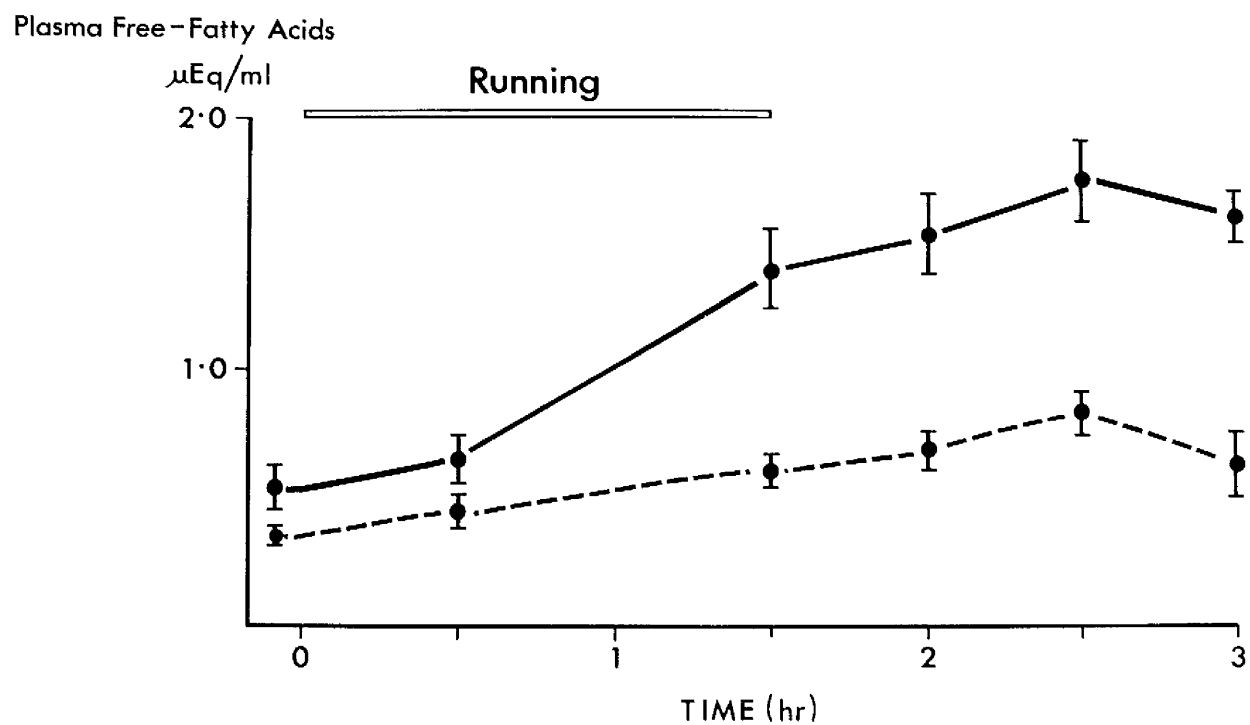


Fig. 3:8 Plasma free fatty acids ( $\mu\text{equiv/ml}$ ) in 9 athletes (—) and 18 untrained subjects (---), (mean  $\pm$  1 S.E.M.).

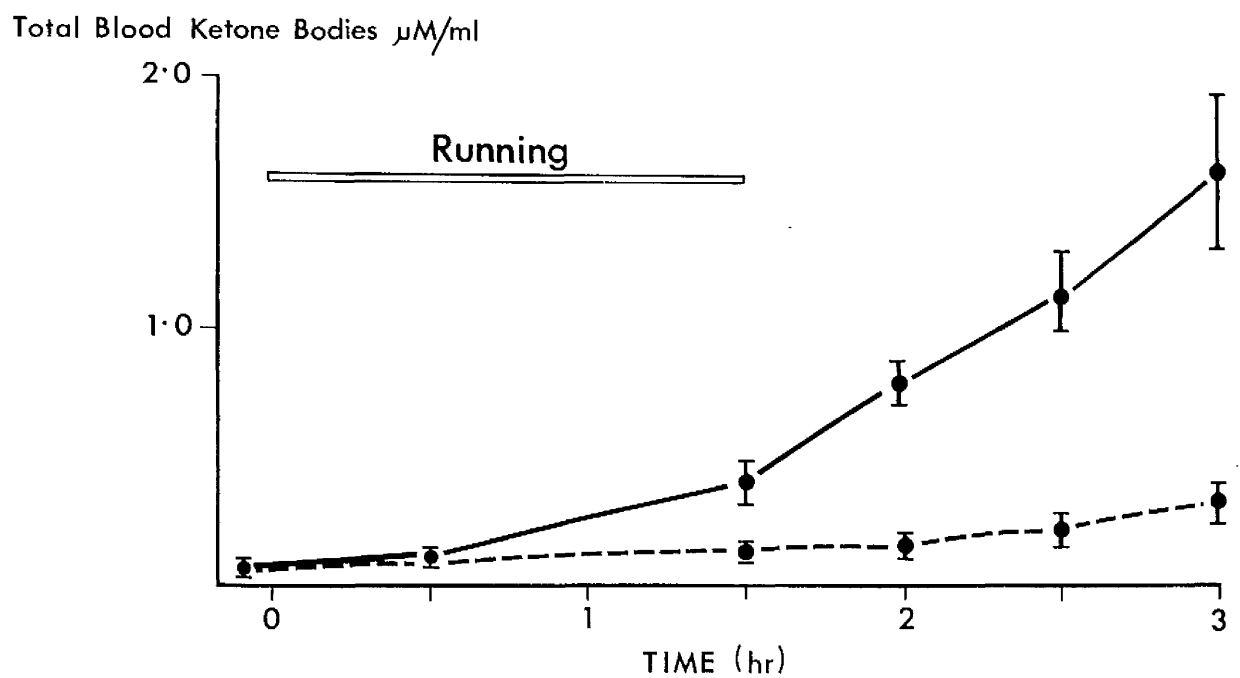


Fig. 3:9 Total blood ketone bodies ( $\mu\text{mole/ml}$ ) in 9 athletes (---) and 18 untrained subjects (—), (mean  $\pm$  1 S.E.M.).

in both groups (range 1.6-2.2) during exercise, but were higher in the untrained subjects (2.8-3.3) compared to the athletes (2.3-2.7) during the recovery period.

### Electrolytes

The plasma concentrations of sodium and chloride increased during exercise in both groups by 6 to 10 mequiv/l, from mean levels at rest of 140 and 102 mequiv/l respectively, and plasma potassium increased by up to 1.0 mequiv/l from a mean level at rest of 4.0 mequiv/l. Plasma bicarbonate fell from a mean level of 22 mequiv/l by 3 to 7 mequiv/l in the 30 min sample, and then returned to original levels. Plasma urea rose slowly during exercise to a level about 20% above the initial reading.

### Discussion

The two groups of subjects showed a considerable difference in running ability, as indicated by their performances, and this was accompanied by clear differences in the physiological and biochemical characteristics that were studied. The two groups were of similar height and weight, but the athletes tended to be slightly older than the untrained group.

This difference resulted from accepting subjects most readily available, the untrained group being students, while some of the athletes were older because those competing in middle and long-distance events usually do so late in their athletic careers.

The trained subjects ran at consistent speeds which they said were appropriate to the time they were asked to run, and, although there was no competition these were high and approximately four-fifths of the world record speeds over comparable distances (Lloyd, 1966). The untrained subjects had difficulty in pitching their speed, and their groups normally started off running too fast and then they would split into ones and twos as they got slower. Despite their greater speeds of running, the athletes had heart rates which remained slower throughout the exercise. The pulses taken before the exercise began showed a wide variation, probably because many of the subjects were anxious about having blood samples taken. The capacity of the circulatory system to adapt to repeated exercise involves increases in haemoglobin and blood volume, and the lower heart rates probably reflect a large heart stroke volume (Wang et al., 1961; Bevegard and Shepherd, 1967).

The athletes had lower gut temperatures during the first hour of exercise than the non-trained individuals, suggesting that the athletes were

dissipating their heat more effectively. This could be achieved with a greater peripheral blood flow or by a greater rate of sweating, probably the latter in view of the higher percentage weight loss of the athletes. Marathon runners have been shown to achieve sweat rates equal to the highest amounts reported for heat-acclimatised men exercising under heat stress (Pugh, Corbett and Johnson, 1967).

The differences in performance, heart rate, weight loss and gut temperature were accompanied by differences in the levels of metabolites related to energy supply.

#### Lactate and Pyruvate

The blood lactate concentration rose by only  $0.18 \mu\text{mole/ml}$  in the athletes, but by  $3.49 \mu\text{mole/ml}$  in the untrained subjects after 30 min exercise. During the following 60 min running period the lactate concentration of the trained subjects only rose slightly. Harris et al., (1968), studying 30 min periods of exercise on an ergometer, found that the lactate concentrations peaked after 10 min but they obtained a plateau if the exercise was more severe. However, the observations of Astrand et al., (1963) on very severe exercise suggest that the lactate concentration will fall if the exercise is continued

for long enough. The increased concentrations of lactate in the initial period of exercise probably reflect a transient hypoxia in the exercising muscles (Harris et al., 1968).

The changes in pyruvate generally paralleled those of lactate in both groups. Lower concentrations of lactate and pyruvate in the blood of athletes during exercise have been observed by several workers (Holmgren, 1956; Holmgren and Strom, 1959; Cobb and Johnson, 1963; Juchems and Kumper, 1968). Thus, athletes either produce less lactate during the early stages of exercise, or they dispose of it more rapidly. Increased disposal could be due to an increased rate of oxidation in muscle, or to an accelerated rate of hepatic gluconeogenesis from lactate. A decreased production of lactate in athletes might be due to the greater blood flow found in contracting muscles of non-athletes and athletes, compared to sedentary subjects (Elsner and Carlson, 1962).

#### Glycerol and Free Fatty Acids

Exercise is accompanied by an increased turnover and oxidation of FFA (Friedberg et al., 1960; Havel et al., 1963), and raised plasma levels have been found in the period of recovery (Carlson and Pernow, 1959). These experiments have shown a steady rise in FFA in both groups during exercise, but whilst

the concentration doubled in athletes, it trebled in the untrained group after  $1\frac{1}{2}$  hr. This difference could be due to either the athletes mobilising less fat or utilising it at a faster rate. The amount of glycerol appearing in the venous blood may be taken as a guide to changes in adipose tissue lipolysis, and as the increase in glycerol during exercise was similar in both groups, it can be assumed that there was no major difference in fat mobilisation between the athletes and non-athletes. The free fatty acid/glycerol ratio was similar in both groups (about 7) before the start of exercise, but was much lower in the athletes (1.5-2.5) compared to the non-athletes (4) during exercise, and this difference was maintained in the recovery period. This suggests that the athletes are able to oxidise fatty acids more effectively than untrained subjects.

#### Ketone Bodies

The relatively low concentrations of acetoacetate and 3-hydroxybutyrate in both groups during exercise suggest that ketone bodies are unlikely to be an important fuel in this situation, because in the resting animal at least, the rate of ketone body utilisation appears to be directly related to their concentration in the blood (Nelson, Grayman and Mirsky, 1941; Bates, Krebs and Williamson, 1968).

This low concentration of ketone bodies during exercise, in contrast to the post-exercise period, may also be important in ensuring an ample supply of free fatty acids (and glucose) because high concentrations of ketone bodies exert both an antilipolytic and hypoglycaemic effect (Bjorntorp and Schersten, 1967; Balasse and Coms, 1968; Senior and Loridan, 1968). The absence of any appreciable rise in ketone body concentration during the post-exercise period in the trained group can partly be explained by the lower FFA concentration, but may also indicate a facilitation of ketone body utilisation.

### Glucose

This was the only metabolite which did not show major differences between the two groups, and this is probably a reflection of its minor role as fuel in the later stages of the type of exercise studied.

### Electrolytes

The slight increases found in plasma sodium and potassium levels have been noticed previously (Keys, 1940; Harris et al., 1968) though in both cases the exercising period was shorter.



Harris also observed an initial rapid fall in plasma bicarbonate occurring within a few minutes of the onset of exercise, and in his subjects who exercised for 30 minutes a subsequent rise in level occurred. the 30 minute sample in these experiments reflected a minimum bicarbonate level which may have already started to rise again.

### Summary

Training of athletes has been shown to affect in a major way the metabolism of fat and carbohydrate during and after exercise. The concentrations of acetoacetate, 3-hydroxybutyrate, free fatty acids, glycerol, lactate, pyruvate, and glucose have been measured in the blood of 9 athletes and 18 non-athlet before, during, and after running for  $1\frac{1}{2}$  hr. Measurements were also made of speed of running, weight loss, and heart rate. There were marked differences in metabolite concentrations between the two groups. The concentrations of lactate and pyruvate did not rise in the group of athletes during the initial period of exercise. The untrained group developed post-exercise ketosis which was associated with high free fatty acid concentrations. The free fatty acid concentrations increased much less in the athletes during exercise, and consequently in the recovery

period they had relatively low ketone body concentration. So, in general, the metabolite concentrations in the blood of athletes deviate less from normal during exercise than those of untrained subjects.

ii. The effect of exercise on an ergometer

The substantial differences between athletes and non-athletes described in the first part of this chapter may be criticised in that the athletes are running faster and performing more work than the other group. In this section the responses of subjects performing at an equivalent work rate on a bicycle ergometer are described.

Methods

Subjects One athlete (age 20, height 173 cm, weight 60. kg) who ran in middle-distance events for Glasgow University, and two untrained subjects (D.M., age 19, height 170 cm, weight 66 kg; N.B. age 19, height 184 cm weight 92 kg ) were studied after they had fasted for 7

Procedure. The subjects exercised on an ergometer set to the same work load for three 10 min periods. Their ventilation, oxygen uptake and carbon dioxide output were measured during the exercise (Jennett, 1968) and heart rates were recorded continuously. Sweat rates were measured in each exercise period by placing previously weighed squares of filter paper on the forearm beneath a plastic sheet. Ear temperatures were recorded every minute with thermistors and the wet and dry bulb temperatures of the room measured with a whirling hygrometer.

Blood samples were taken before the exercise, at the end of each 10 min period and at three subsequent 30 min intervals. They were analysed for lactate, pyruvate, glucose, acetoacetate,  $\beta$ -hydroxybutyrate, glycerol and plasma free fatty acids and electrolytes (chapter 1), at the end of exercise and 1 hr later, serum was taken for thyroxin assay.

### Results

The heart rates and ventilating responses of the athlete and the unfit subject D.M. are shown in Fig. 3. Unfit subject N.B. showed ventilatory and heart rate changes similar to D.M. The heart rate of the athlete showed a smaller rise during exercise than the other two and returned to within 15 beats of resting level 2 min after stopping exercise whereas the heart of the unfit subjects remained above 115 beats/min in the same period. Despite the same work load during the first two 10 min periods of exercise ( $1\frac{1}{2}$  kiloponds at 32-33 Kph) the unfit subjects showed a relative hyperventilation as indicated by the high  $\dot{V}CO_2$ . In the last period of exercise the fit subject exercised slightly faster ( $1\frac{1}{2}$  kiloponds at 35 Kph) and his rate of ventilation increased to the same level as that of the unfit subjects. Each subject showed the highest sweat rate in the last period of exercise and the athlete sweated more and

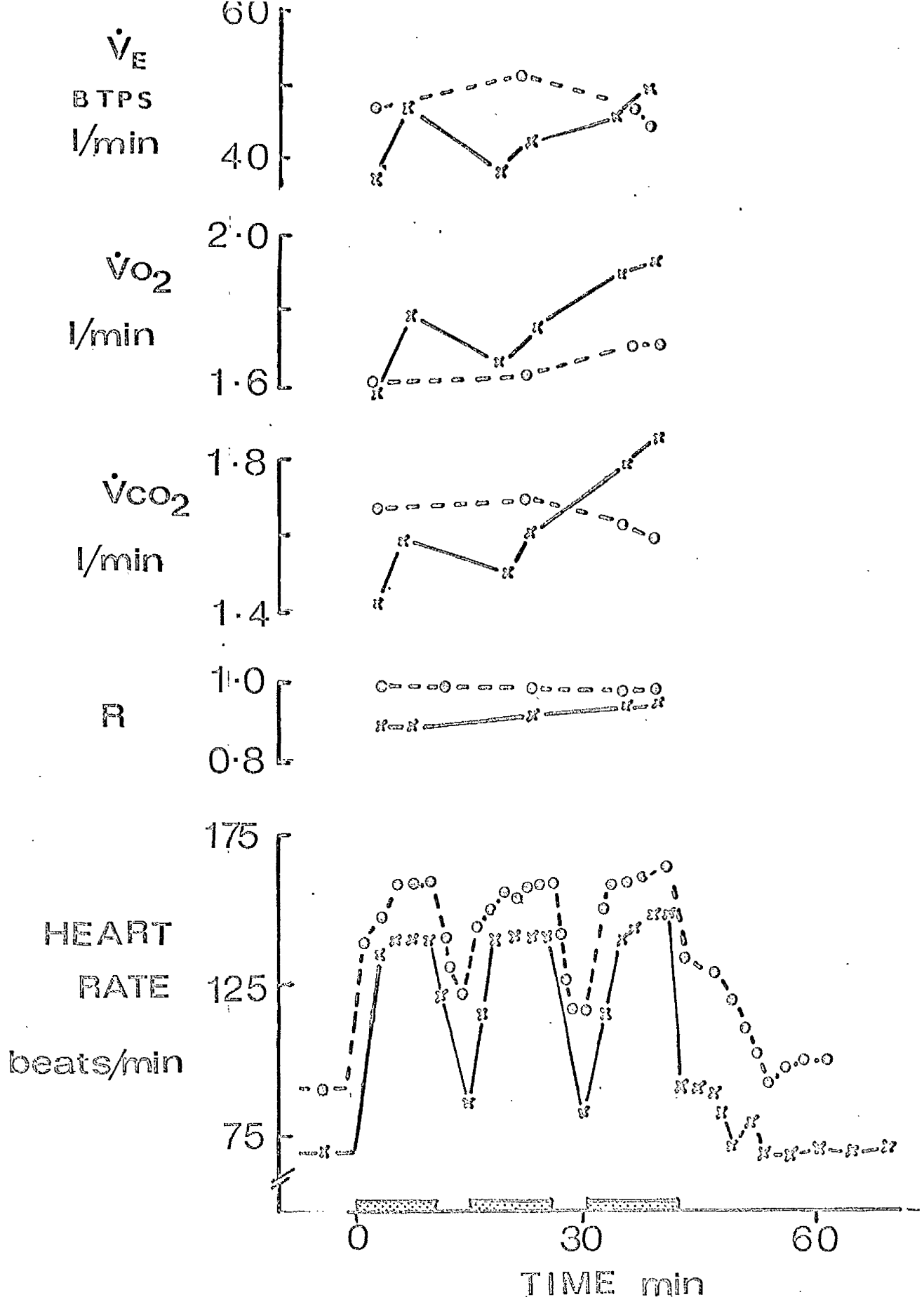


Fig. 3:10 Ventilatory responses and heart rates of an athlete (x—x) and a non-athlete (o—o) during three 10 min periods of exercise on a bicycle ergometer.

lost more weight during the exercise ( Table 3:2). The athlete also showed a smaller rise in temperature in the first period of exercise ( $0.65^{\circ}\text{C}$ ) than unfit subject D.M. ( $1.0^{\circ}\text{C}$ ).

The athlete showed no change in blood lactate and pyruvate levels in the exercise period, whereas the unfit subject D.M. showed substantial increases( Figs. 3:11 and 3:12). Subject N.B. also showed increases in pyruvate (rest  $0.064\text{ }\mu\text{mole/ml}$ , peak  $0.193\text{ }\mu\text{mole/ml}$ ) and lactate(rest  $0.68\text{ }\mu\text{mole/ml}$ , peak  $2.48\text{ }\mu\text{mole/ml}$ ). The unfit subjects also showed larger increases in FFA, glycerol and total ketone bodies than the athlete (Figs 3:13,14 and 15 ). The peak values in subject N.B. were glycerol  $0.130\text{ }\mu\text{mole/ml}$ (50 min), FFA  $0.81\text{ }\mu\text{equiv/m}$  and total ketone bodies  $0.280\text{ }\mu\text{mole/ml}$  (at 2 hr) ; glucose concentrations followed a similar pattern in ea subject falling slightly during the initial part of the exercise (Fig. 3:16 ).Plasma sodium, potassium and chloride showed small increases in all the subjects, while plasma bicarbonate decreased  $3\text{--}5\text{mequiv/L}$  during exercise. The free thyroxin index decreased slightly in the fit subject during exercise but increased in unfit subject D.M. (Table 3:3 ).

Subject	Humidity (%)	Total wt. loss (% body wt.)	Sweat rate (mg/10cm <sup>2</sup> /10 min)			
			1st 10 min	2nd 10 min	3rd 10 min	after exercise
J.McH.	44	1.50	20	82	140	23
D.M.	40	0.63	2	39	59	17
N.B.	37	0.79	5	52	67	10

Table 3:2 Sweat rates in an athlete (J.McH.) and two unfit subjects during exercise on an ergometer.

Subject		P.B.I. ( $\mu$ g%)	Free thyroxine Index
J.McH.	before exercise end of exercise 1 hr. later	4.3 4.4 4.3	4.86 4.66 4.86
D.M.	before exercise end of exercise 1 hr. later	5.1 4.5 4.8	5.30 5.44 5.66

Table 3:3 Plasma bound iodine (P.B.I.) and free thyroxine indices in an athlete (J.McH.) and an untrained subject (D.M.) after 30 min of exercise on an ergometer.

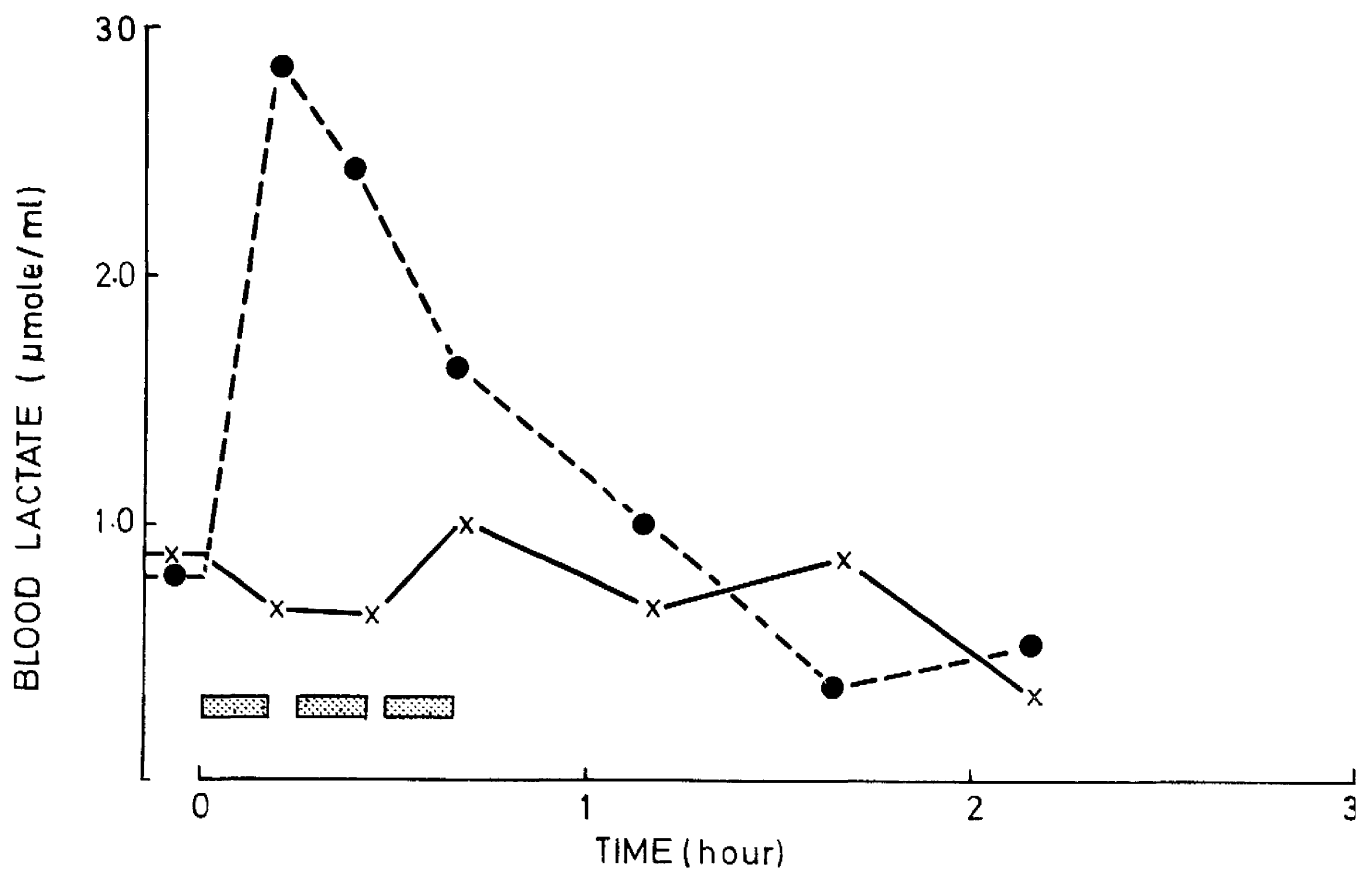


Fig. 3:11 Blood lactate ( $\mu\text{mole/ml}$ ) in one trained athlete (x—x) and one untrained subject (● - - - ●). The stippled bars represent the period of exercise.



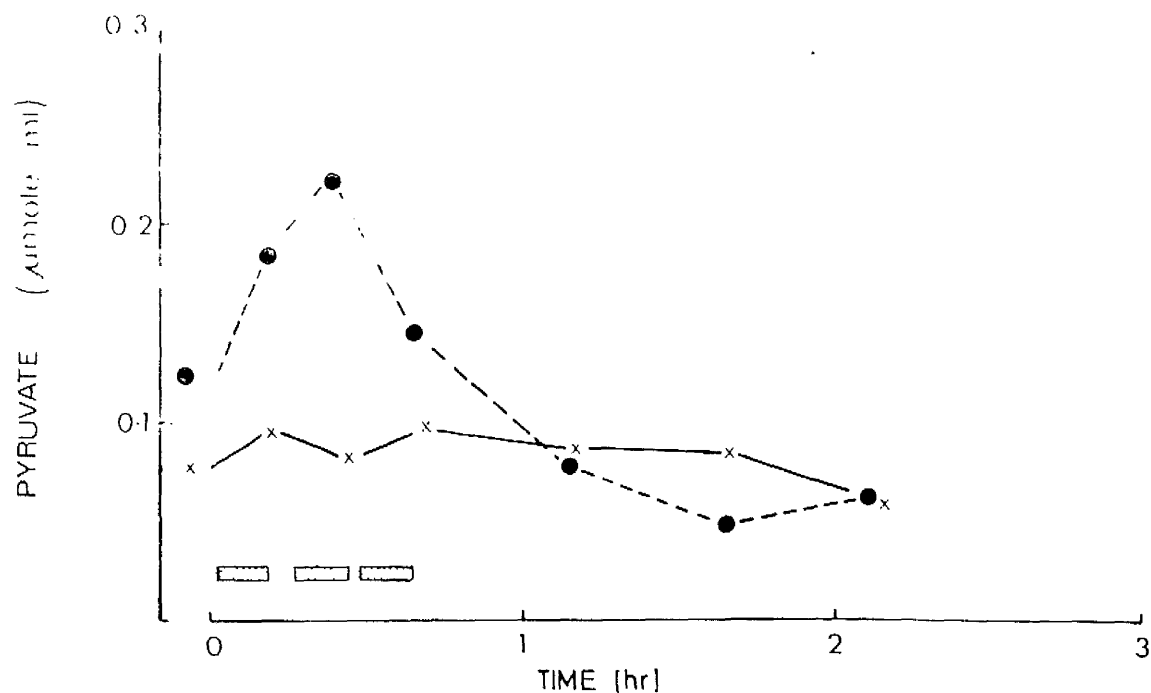


Fig. 3:12 Blood pyruvate ( $\mu\text{mole/ml}$ ) in one trained athlete (x—x) and one untrained subject (•- - -•). The stippled bars represent the period of exercise.

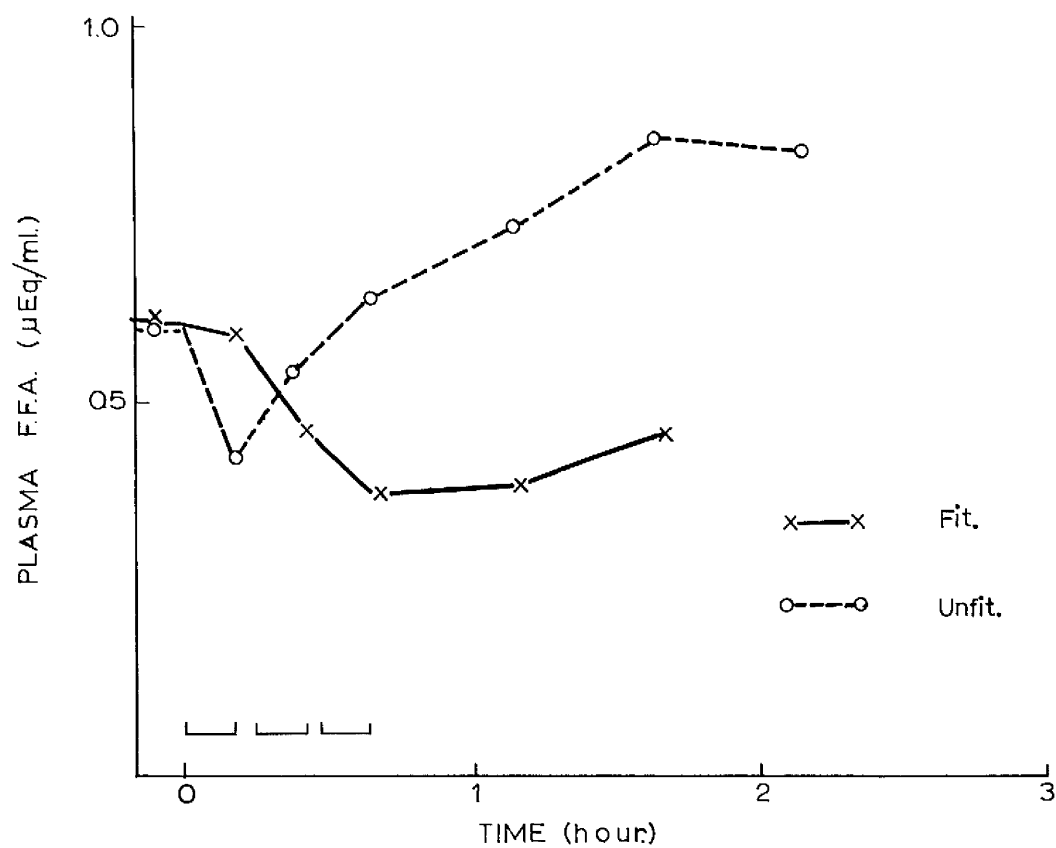


Fig. 3:13 Plasma free fatty acids ( $\mu$ equiv/ml) in one trained athlete (x—x , fit ) and one untrained subject (o — — — o , unfit ). The bars represent the period of exercise.

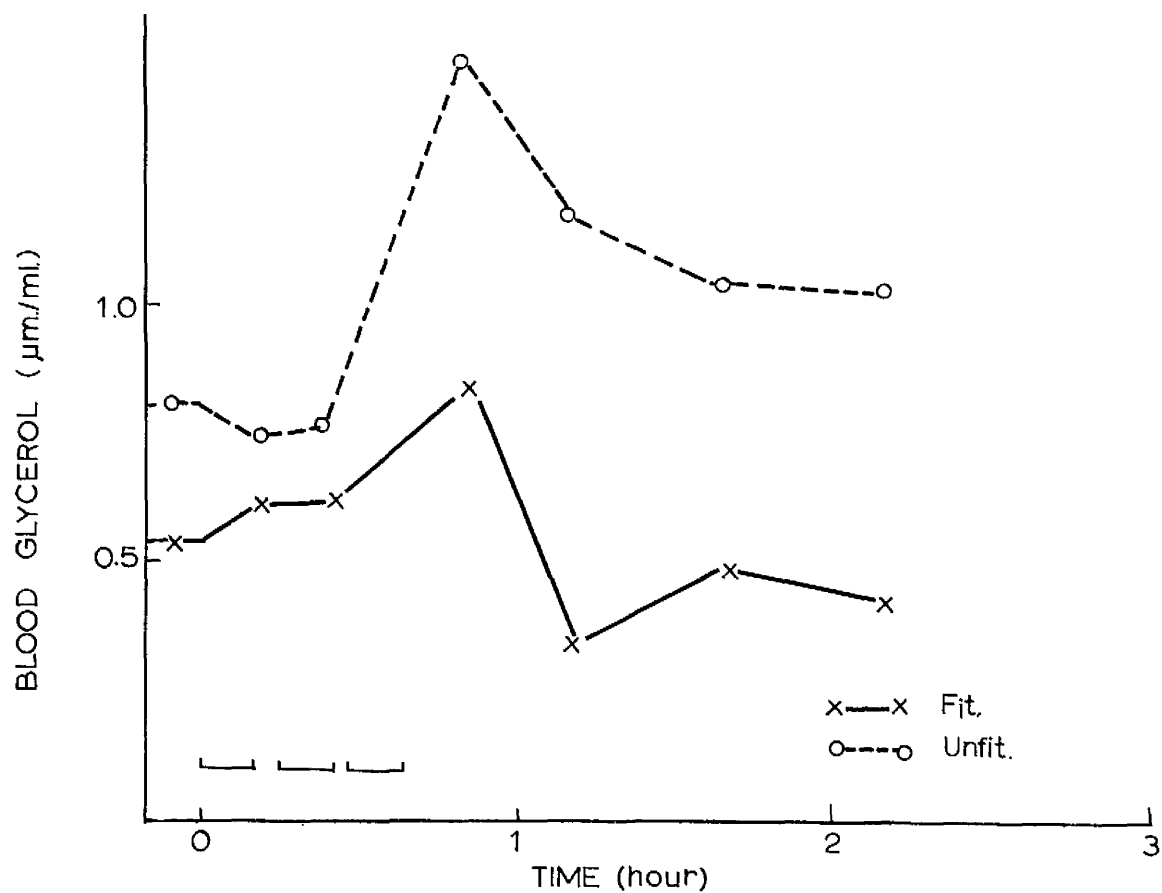


Fig. 3:14 Blood glycerol ( $\mu\text{mole/ml}$ ) in one trained athlete (x—x ,fit ) and one untrained subject (o—o , unfit ). The bars represent the period of exercise.

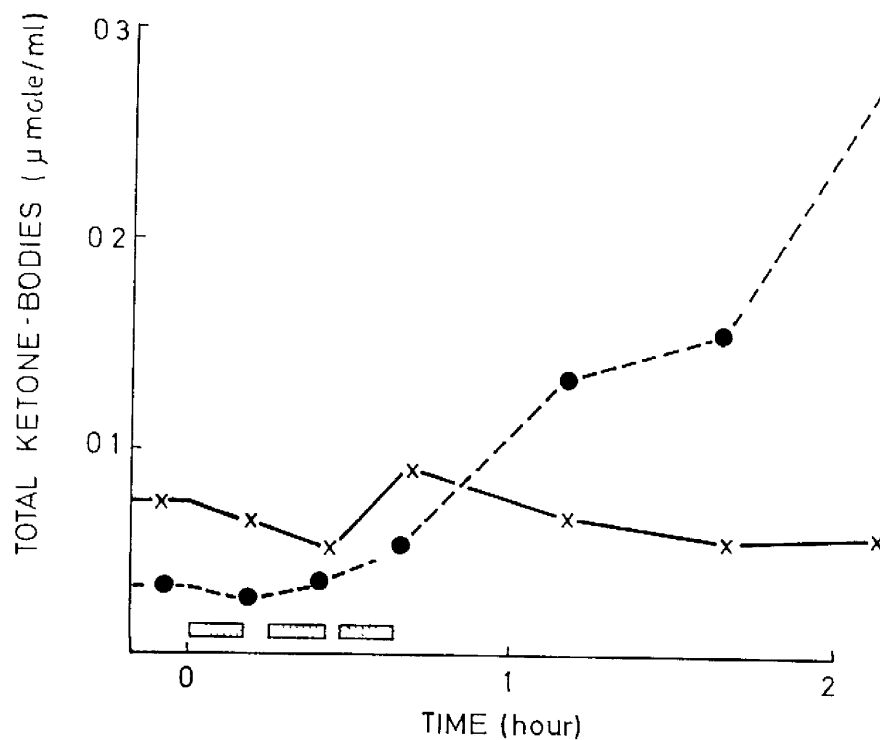


Fig. 3:15 Blood ketone bodies ( $\mu\text{mole/ml}$ ) in one trained athlete (x—x) and one untrained subject (o—o). The stippled bars represent the period of exercise.

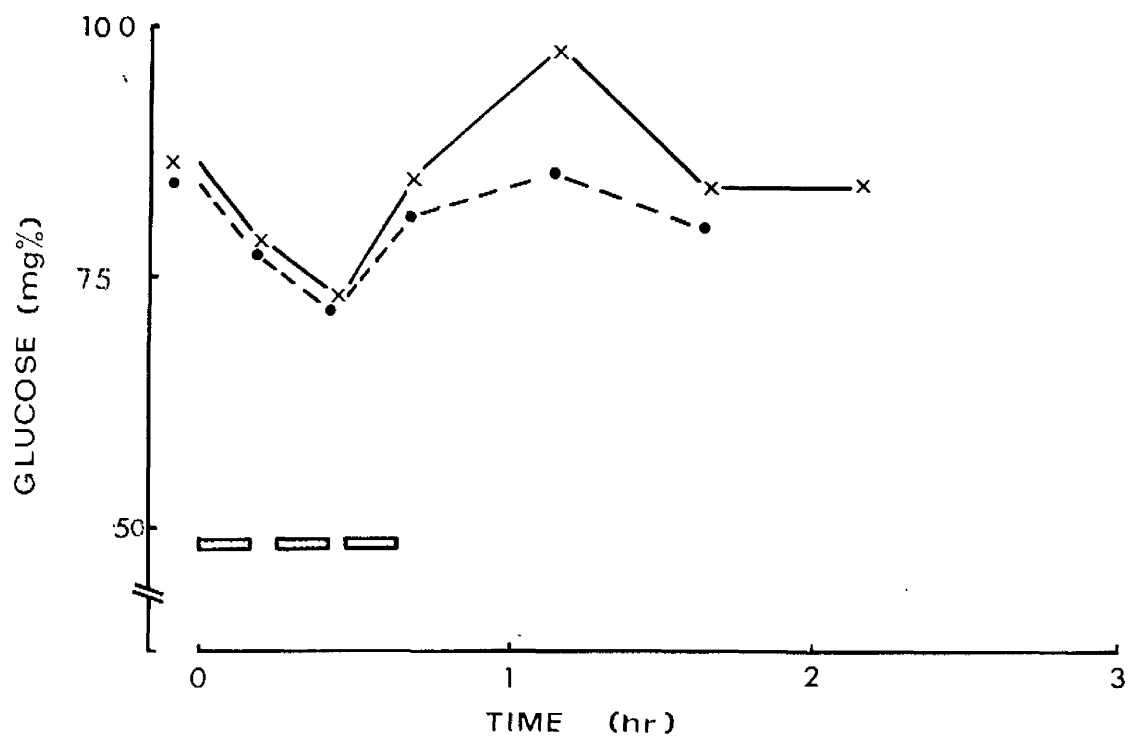


Fig. 3:16 Blood glucose (mg %) in one trained athlete (x—x) and one untrained subject (o—o). The stippled bars represent the period of exercise.

## Discussion

The differences between the athletes and untrained subjects observed in the previous section might have been due to the two groups performing at different work rates. Oxygen consumption during running is linear related to speed (Menier and Pugh, 1968) and in that investigation the mean oxygen intakes of the athletes must have been about 50 ml/kg/min (athletes) and 30ml/kg/min (untrained subjects). In this exercise on the ergometer the work rates were similar in each subject and the average oxygen intakes were 29 ml/kg/min (athlete), 25 ml/kg/min (D.M.), 28 ml/kg/min (N.B.).

Despite similar work loads and oxygen intakes the physiological responses of the athlete ( heart rate, temperature change, weight loss) were different from those of the other subjects, and the observations agreed with those made during the running experiments.

The biochemical changes were also in accord with those made previously though the timing of the blood samples emphasised the changes occurring early during exercise. The lactate concentration reached a peak before the pyruvate concentration, as noted by Harris et al.(1968) and the initial fall in plasma FFA observed by Carlson and Pernow (1961) and by Havel, Naimark and Borchgrevink (1963) was also made apparent.

Although the exercise was not severe or prolonged, marked elevations of ketone bodies still occurred after the exercise in the unfit subjects.

The small drop in free thyroxin in the athlete and the increase in the unfit subject follow the changes in plasma FFA observed in these subjects. This substantiates the findings of Radomski, Britton and Schonbaum (1967) who described a close correlation between free thyroxin and FFA in rats and men after exercise. The increased free thyroxin in the untrained subject might play a part in the greater heat production observed, though this must be partly explained by the lower sweat rate and smaller loss of body weight.

### Summary

One athlete and two untrained subjects exercised for three 10 min periods at the same work load on a bicycle ergometer. During and after this period of moderate exercise the changes in blood metabolic levels were qualitatively similar to those described in the first part of this chapter. The athlete showed smaller changes than the other two subjects and did not develop post-exercise ketosis.

## CHAPTER 4

### i. Fitness, fatness, and post-exercise ketosis

In Chapter 3 it was shown that subjects not in regular athletic training develop ketosis after strenuous exercise much more readily than athletes, but it is not known whether this negative relationship between fitness and capacity to develop ketosis applies throughout a wide range of fitness or is obvious only at extremes.

Post-exercise ketosis probably arises from an excessive use of fat over carbohydrate as an energy source, and as there is a negative correlation in men between obesity and fitness (Miller and Blyth, 1955; Sloan, 1969) the possibility exists that ketosis could be related to body fat content. In order to investigate the relationships between fitness and fatness, and levels of blood ketones and free fatty acids developed after exercise, these factors have been studied in a group of young men with a wide range of fitness.

### Methods

Subjects      Twenty-one healthy male subjects, aged 19-23,



were studied. Their participation in sporting activities ranged from nil to University representation in running. The subjects were volunteers from a University Hall of Residence, and from a Glasgow running club (Garscube Harriers).

Fitness Each subject performed the Harvard Step Test (Brockett et al., 1956). He stepped on to and down from a platform 20 in high, 30 times a minute for 5 min, or until fatigue caused him to slow for more than 15 sec, or stop. Immediately after the exercise, the subject was asked to sit down and his pulse was counted from 1-1½ min after the exercise by two observers. The Harvard Fitness Index (F.I.) was calculated from the duration of exercise and the single post-exercise pulse count (Weiss and Phillips, 1954) from the formula:

$$F.I. = \frac{\text{duration of exercise (sec)} \times 100}{5.5 \times (\text{pulse count 1-1½ min after exercise})}$$

Sloan (1959) reports that the conventional classification of fitness on the basis of this test is F.I. < 50: poor, F.I. 50-80: average; and F.I. > 80: good.

Fatness The fat content of the body, expressed as a percentage of body weight, was estimated from body density derived from skinfold measurements with the Harpenden and H.N.L. calipers (Best, 1954) using the

formula (Sloan, 1967):

$$D = 1.1043 - 0.001327x_1 - 0.001310x_2$$

where, D = density (g/ml)

$x_1$  = vertical skinfold in anterior midline of  
right thigh, midway between inguinal ligament  
and top of patella (mm)

$x_2$  = skinfold running downward and laterally from  
inferior angle of right scapula (mm).

Body fat was then calculated by Brožek's formula  
(Brožek et al., 1963 ):

$$F = 100(4.570/D - 4.142)$$

where, F = fat (percent of body weight)

D = density (g/ml).

Post-exercise ketosis      The subjects ran at a steady  
pace for 30 min on an outdoor track, covering distances  
of 7.0-8.0 km. No food had been consumed in the previous  
5-6 hr. Venous blood samples were taken before the run,  
and at 1 hr, and 2 hr afterwards. They were analysed  
for acetoacetate, 3-hydroxybutyrate and the plasma for  
free fatty acids.

## Results

The fitness indices ranged from 55-170 and body fat percentages from 5.3 - 24.0 %.

The total blood ketones after exercise also showed considerable variation, ranging from 0.081 - 0.434  $\mu$ mole/ $\pi$ . Free Fatty acids ranged from 0.85 - 2.90  $\mu$ equiv/ml. The results for each subject are given in Table 4:1, and the relations between the fitness indices ( $\log_{10}$  scale), percent body fat and blood ketones are shown in Figs 4:1, 2 and 3. The relation between fitness ( $\log_{10}$  scale) and plasma free fatty acid levels is shown in Fig. 4:4. There was a highly significant negative correlation between the maximum level of ketosis developed 1 - 2hr after exercise, and the  $\log_{10}$  Fitness Index ( $r = -0.60$ ,  $p < 0.005$ ) although there was also evidence of negative correlation between fitness ( $\log_{10}$  F.I. ) and percentage of body fat ( $r = -0.44$ ,  $p < 0.05$ ). There was a significant positive correlation between fitness ( $\log_{10}$  F.I.) and plasma free fatty acid levels 1 hr after exercise ( $r = +0.54$ ,  $p < 0.025$ ), but not 2hr after exercise. There was no significant correlation ( $r = 0.27$ ,  $p > 0.10$ ) between the extent of post-exercise ketosis and the degree of obesity in the subjects studied (Fig. 4:3).

TABLE 4:1

Sub- ject	Age  yr	F.I.	Body fat  %	total blood ketone bodies ( $\mu$ mole/ml) after running		plasma free fatty acids ( $\mu$ equiv/ml) after running	
				1 hr	2 hr	1 hr	2 hr
1	35	80	24.1	0.102	0.112	0.65	1.2
2	23	71	9.4	0.190	0.412	2.25	2.9
3	23	81	8.3	0.116	0.082	1.21	1.6
4	19	88	5.3	0.092	0.090	1.81	1.6
5	22	74	20.0	0.191	0.375	1.86	1.7
6	19	80	8.3	0.063	0.081	1.71	2.1
7	19	78	9.0	0.143	0.183	1.57	2.5
8	20	84	12.0	0.102	0.047	1.52	1.6
9	22	81	6.8	0.081	0.252	1.36	0.8
10	20	84	12.0	0.178	0.347	0.90	2.1
11	24	75	7.3	0.095	0.312	0.21	1.7
12	23	84	7.3	0.222	0.398	0.31	2.3
13	20	63	15.9	0.112	0.303	0.29	2.0
14	30	55	19.1	0.128	0.432	0.86	2.5
15	22	72	9.5	0.274	0.371	1.22	2.3
16	18	58	9.0	0.434	0.351	0.13	2.1
17	24	170	6.2	0.088	0.081	2.31	2.1
18	21	121	5.8	0.047	0.083	1.50	1.3
19	19	105	7.2	0.196	0.245	2.06	1.8
20	24	99	10.9	0.163	0.201	1.25	1.6
21	22	81	15.8	0.404	0.432	1.70	1.4

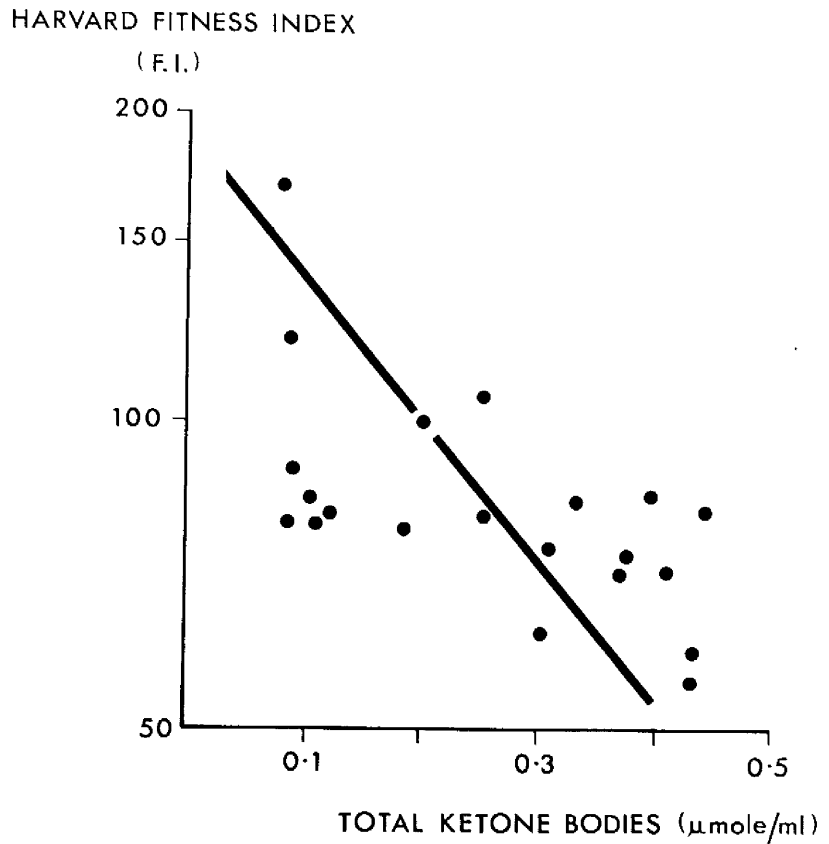


Fig. 4:1 The relation between total blood ketone bodies (μmole/ml) in 21 subjects, highest result 1- after running for 30 min, and each subject's Harvard Fitness Index. The vertical axis is plotted as a  $\log_{10}$  scale because of the wide range of fitness. The regression line fits the formula : Ketone Bodies =  $1.74 - 0.774 \log_{10}$

HARVARD FITNESS INDEX  
(F.I.)

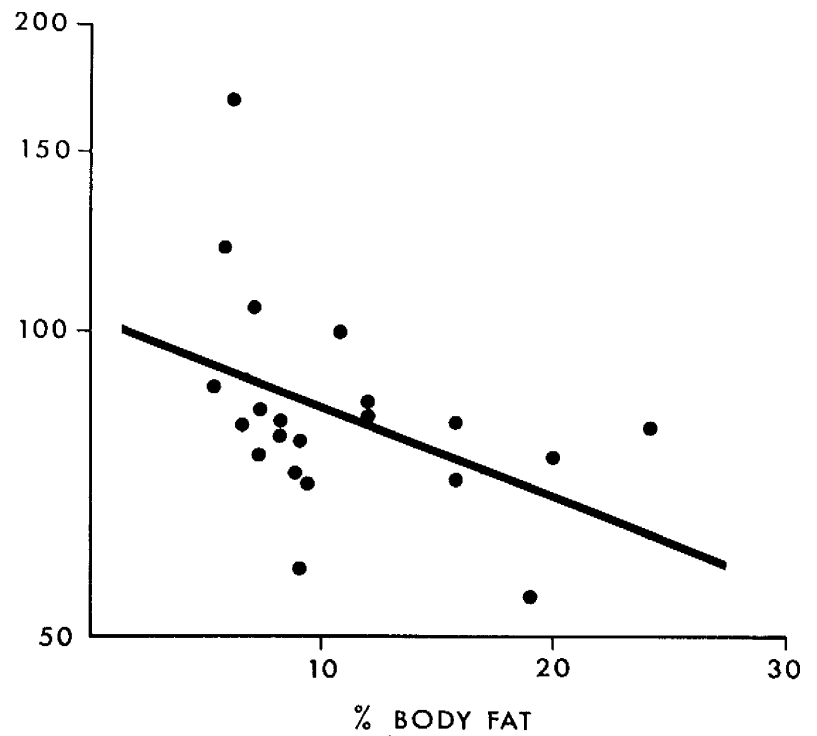


Fig. 4:2 The relation between body fat ( % of total body weight) and the Harvard Fitness Index (  $\log_{10}$  scale) of the 21 subjects. The regression line fits the formula :  $\text{Log}_{10}\text{F.I.} = 2.01 - 0.0089\%$

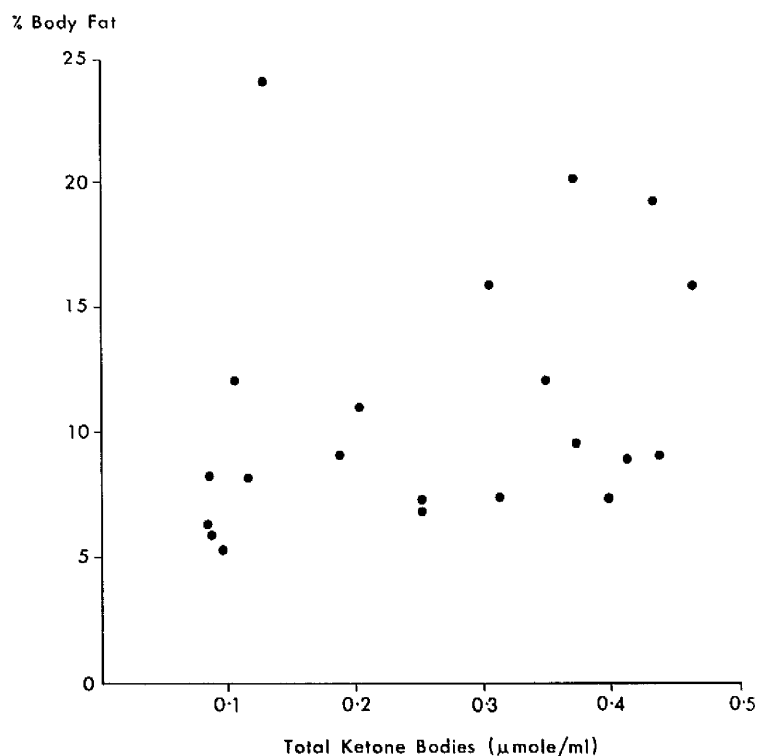


Fig. 4:3 The relation between total blood ketone bodies ( $\mu\text{mole/ml}$ ), highest result 1-2 hr after running for 30 min, and body fat (% of total body weight) in the 21 subjects. This relationship is not significant ( $r = 0.27$ ;  $p > 0.10$ ).

HARVARD FITNESS INDEX  
(F.I.)

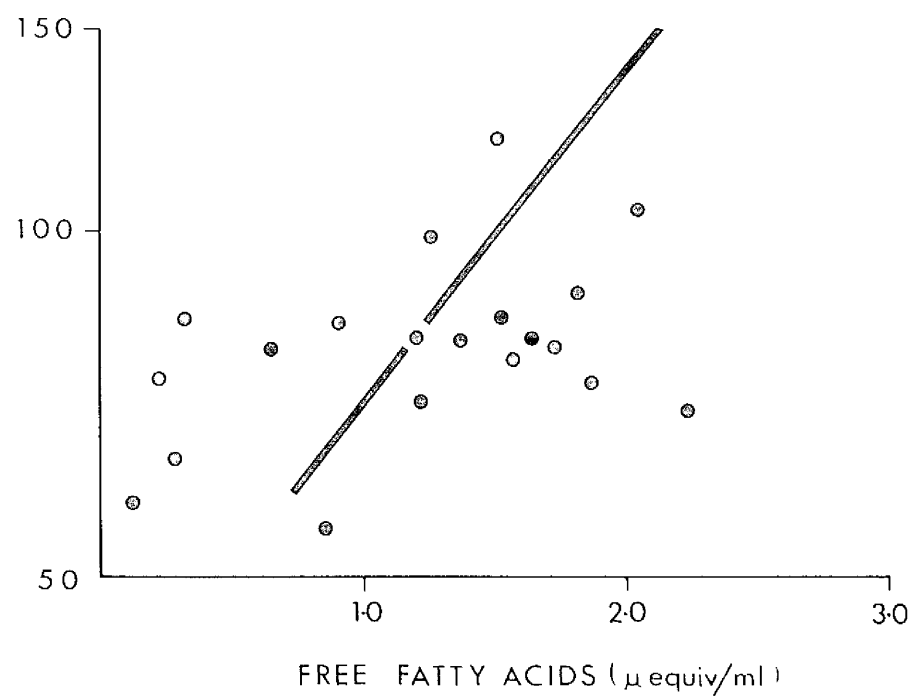


Fig. 4:4 The relation between free fatty acids ( $\mu$ equiv/ml) 1 hr after exercise and the Harvard Fitness Index ( $\log_{10}$  scale). The regression line fits the formula :  $\text{FFA} = -5.19 + 3.37 \log_{10} \text{F.I.}$



## Discussion

Fitness has been assessed by the Harvard Step Test because this is widely considered to be a useful test of fitness and the results of a step test bear comparison with studies on an ergometer or treadmill (Allen et al, 1967). The test was not modified for individual differences in height, weight or leg length as evidence suggests that the correlations of these factors with F.I. are not significant (Fletcher, 1960; Ishiko, 1967; Keen and Sloan 1958), although there is evidence of an effect of weight in some observations (Mahadeva, Passmore and Wolff, 1953). Obesity was assessed by skinfold measurements as these provide a good relationship with percent body fat (Consolazio, Johnson and Pecora, 1963) and the techniques and formulae used have been found to give satisfactory results (Brožek et al., 1963; Sloan, 1967; 1969).

The period of  $\frac{1}{2}$  hr. of exercise was chosen because observations reported in the next chapter had indicated that it was sufficient to result in ketosis in untrained subjects. There is a significant correlation between F.I. and middle-distance running ability (Ishiko, 1967) and, more importantly it was a time the untrained subjects were prepared to run at a relatively steady rate

These results have supported some previous observations suggesting fitness has a negative correlation with obesity (Miller and Blyth, 1955; Welch et al., 1958; Sloan, 1969) and have gone further to indicate that there is a better relationship (negative correlation with  $p < 0.005$ ) between fitness (expressed as  $\log_{10} \text{F.I.}$ ) and the development of post-exercise ketosis. As obesity and post-exercise ketosis are not significantly correlated, it would appear that the levels of ketosis, the accumulation of which depends on excessive free fatty acid mobilisation, are nevertheless not dependent on the extent of fat stores. Exercise increases the rate of lipolysis (Havel, Naimark and Borchgrevink, 1963), and differences in free fatty acid levels in the post-exercise period between athletes and non-athletes have been observed by Cobb and Johnson (1963). They considered, however, that this difference was likely to be related to the longer duration of exercise of the physically active group in their investigations, rather than a difference in the rate of fat metabolism. In this present study, however, the subjects ran for similar periods and speeds, and therefore the correlation ( $r = +0.54$ ,  $p < 0.025$ ) observed between fitness and free fatty acid levels 1 hr after running indicates a probable difference in free fatty acid mobilisation rates. The investigations reported in Chapter 3 showed that if the athletes and

untrained subjects were allowed to choose their own running speed, the fatty acid levels did not show this difference and indeed the levels of the athletes were lower.

### Summary

The fitness index (F.I.) assessed by the Harvard Step Test, and body fat (expressed as a percentage of body weight) were determined on 21 young men. The extent to which they developed ketosis after a half-hour run was measured at the end of the run, and 1hr, and 2 hr later. Plasma free acids were also measured at these times.

Those with higher fitness indices tended to be leaner subjects. There was a better correlation, however, between the subjects' fitness and the degree of post-exercise ketosis, the less fit subjects developing a greater degree of ketosis, and also between fitness and the levels of free fatty acids one hour after exercise. There was no significant correlation between body fat and post-exercise ketosis, and a wide range of ketosis was developed in the subjects with very similar percentages of body fat. It would, therefore, appear that ketone production after exercise can act as a useful index of an individual's fitness.

ii. The effect of training on post-exercise ketosis.

Several of the subjects who offered to run were athletes who were, at the time, unfit and out of training either because the season was just starting or because they were then returning to athletics after a period of inactivity. Investigations on these subjects suggested that the ability to maintain low levels of ketone bodies after exercise was an effect of training rather than, for instance, a genetic endowment associated with running ability.

Methods

Two athletes who were out of condition ran for 30 min or 90 min in company with (i.e. at the same speed and for the same distance) a well-trained athlete. Each member of the pair of fit and unfit athletes belonged to the same running club and had previously been of an equivalent standard. Venous blood samples were taken and analysed for acetoacetate and 3-hydroxybutyrate.

Results

Of the two subjects who ran for 90 min the athlete who was out of training developed a marked post-exercise

ketosis (Fig. 4:5) while the trained athlete did not. The other pair ran for 30 min and again the unfit member of the pair showed an increase in ketone bodies of  $0.230 \mu\text{mole/ml}$  of blood 2 hr after the end of the run while his team mate showed no change at all.

### Discussion

Athletes who were out of training showed similar metabolic responses to exercise as non-athletes and this suggests that the training an athlete carries out is producing alterations in his metabolic control mechanism.

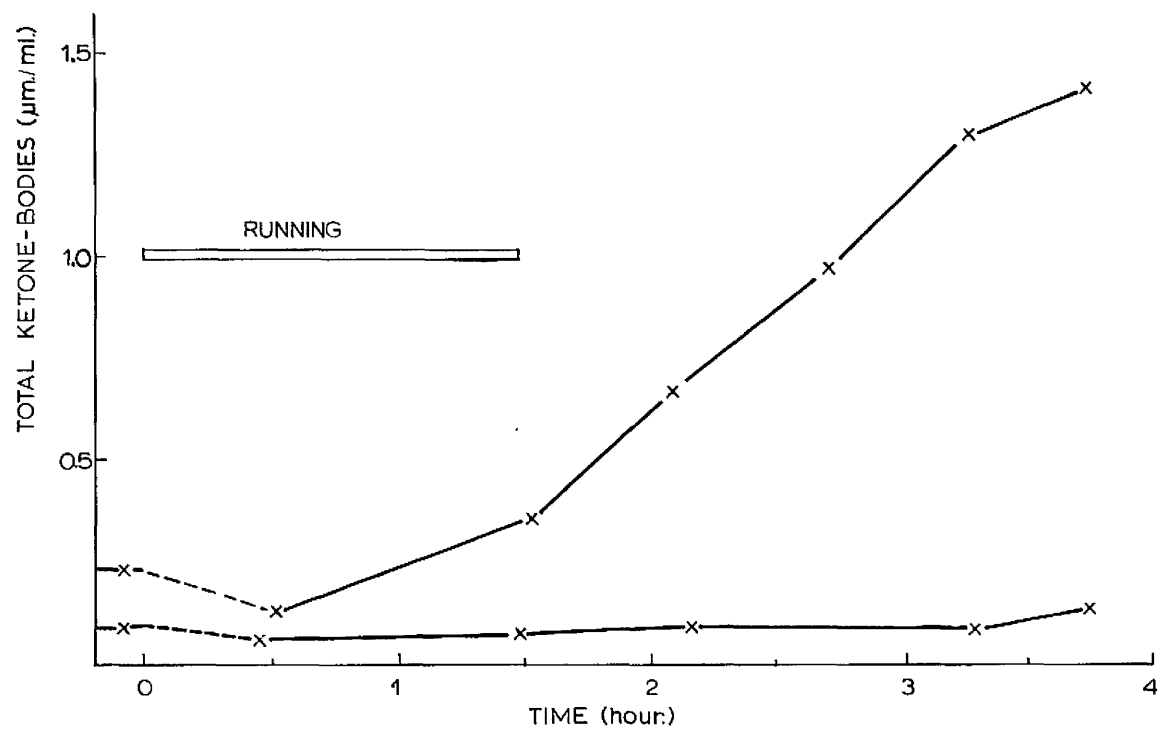


Fig. 4:5 The effect of 1½ hr of running on levels of ketone bodies (μmole/ml) in two athletes , one in peak condition (lower line) and one who has only just started training after an interval 6 months (upper line).

## CHAPTER 5

### i The effect of different durations of running.

Previous studies of post-exercise ketosis have involved relatively mild degrees of exercise, carried out for extended periods. Courtice and Douglas (1936) walked 16.1 km, the subjects in Passmore and Johnson (1958) walked 16.7 km, and those in Galvin, Harris and Johnson (1968) walked 14.5 km. The investigations described in Chapter 3 also involved a considerable duration of exercise, though more severe, and it was therefore of interest to investigate shorter periods of running.

### Methods

Five subjects, University students of varying degrees of fitness, ran for two or three periods of  $\frac{1}{2}$  hr, 1 hr or  $1\frac{1}{2}$  hr on different occasions. In addition one subject ran for the same length of time on two occasions. The exercise was carried out at least 4 hr after any previous food and for each individual at the same time of day. Blood samples were taken at appropriate intervals and analysed for acetoacetate and 3-hydroxybutyrate, and, in some subjects, for plasma FFA.

The Fitness Index was calculated from performance in the Havard Step Test as described in Chapter 4.

### Results

The subject (A.McM.), who ran for two 1 hr periods, developed a similar degree of ketosis after the exercise on each occasion (Fig. 5:1).

The results of the different periods of running are shown in Table 5:1. In the most unfit subject  $\frac{1}{2}$  hr of exercise was sufficient to raise the level of ketone-bodies substantially and a longer period of running merely produced a greater ketotic response. The level immediately after the exercise was greater in the longer period of running. In the other subjects a longer period of running was required to produce raised levels of ketone bodies.

In subject 1 of table 5:1 blood samples were taken for up to 8 hr after running. The peak ketotic response occurred  $4\frac{1}{2}$  hr after running for 1 hr but the level was still rising 8 hr after the longer run (Fig. 5

FFA were measured after 30 min periods of running. Subjects 1, 2 and 4 showed peak FFA levels 2 hr after the exercise whereas subjects 3 and 5 showed a peak level immediately after the exercise.



Subject	Fitness Index	Duration of run (min)	Total Ketone Bodies ( $\mu$ mole/ml) at the following times after exercise (min)			
			Rest	30	60	90 120
1	71	30	0.055	-	0.190	0.360 0.412
		60	0.270	0.380	0.540	0.720 0.720
		90	0.600	-	0.800	0.940 1.160
2	80	30	0.080	-	0.102	0.130 0.112
		90	0.350	0.360	0.475	0.600 -
3	88	30	0.100	-	0.092	- 0.090
		90	0.101	0.145	0.382	0.358 0.363
4	99	30	0.120	-	0.163	- 0.201
		90	0.370	0.660	0.910	1.250 1.410
5	170	30	0.091	-	0.088	- 0.081
		90	0.300	0.220	0.210	0.208 -

Table 5:1 Total blood ketone bodies in five subjects of varying fitness after running for different periods of time.

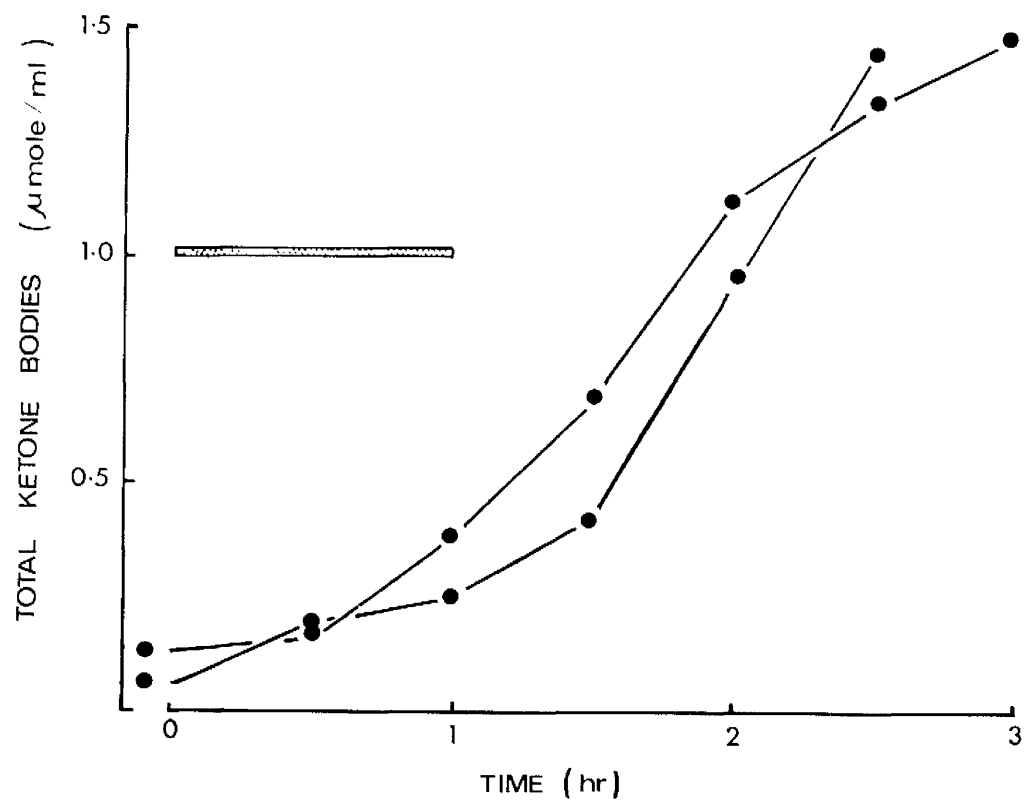


Fig. 5:1 Total blood ketone bodies ( $\mu$ mole/ml) in the same subject on two separate occasions after running for 1 hr.

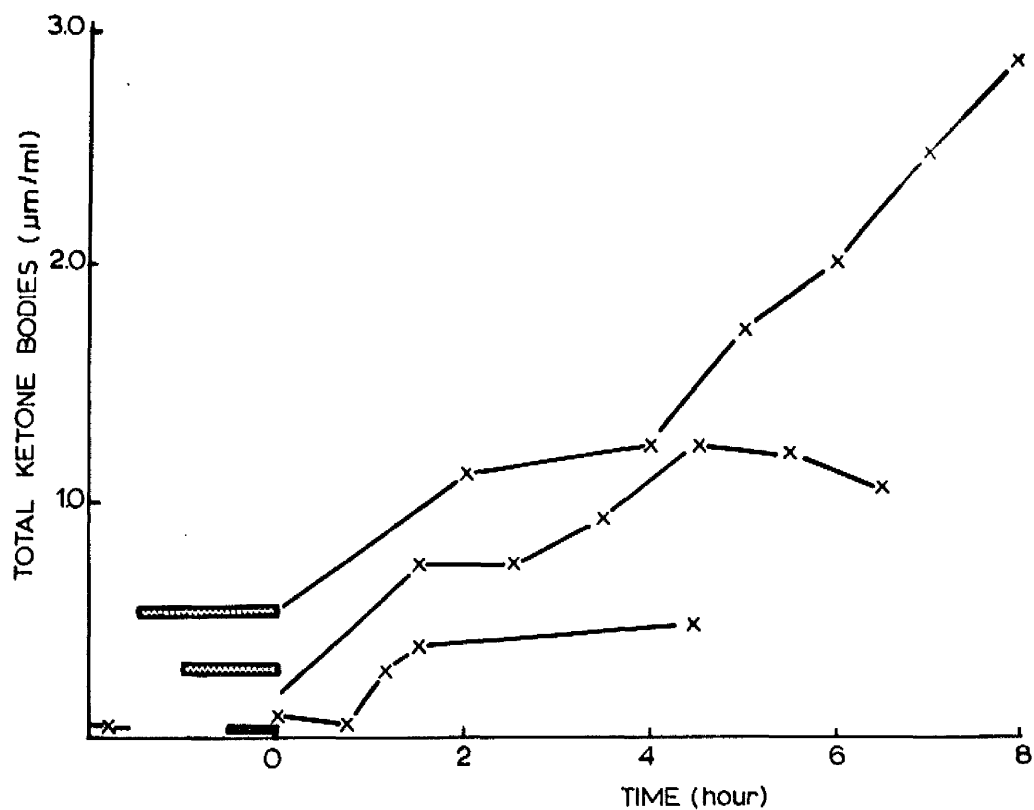


Fig. 5:2 Total blood ketone bodies ( $\mu$ mole/ml, in the same subject after running for three different periods of time ( $\frac{1}{2}$ , 1, and  $1\frac{1}{2}$  hr) on separate occasions.

## Discussion

These results indicate that fairly short periods of exercise suffice to produce increased levels of ketone-bodies. Indeed in a later investigation one subject showed an increase in ketone bodies from a resting level of  $0.091 \mu\text{mole/ml}$  to  $0.375 \mu\text{mole/ml}$   $2\frac{1}{2}$  hr after running about 600 metres at a speed of 11 km/hr. This rise was coupled with a large increase in plasma FFA levels after the exercise and it seems probable that if the stimulus is sufficient to cause an increased rate of lipolysis, then raised ketone body levels will ensue.

The capacity of the subjects 3 and 5 to maintain lower levels of ketone bodies seemed to be related to the timing of the rise in plasma FFA rather than the size of this rise. The results in Chapter 4 also suggest that the fitter subjects show an earlier peak in FFA and this may be caused by the sharper cut off in growth hormone secretion in athletes as is reported in Chapter 8

The higher levels of ketonaemia observed immediately after the longer exercise period compared with the levels in samples taken at the same time (i.e.  $1\frac{1}{2}$  hr) after the start of the 30 min exercise suggests that ketone bodies are not being used as a fuel source as rapidly as they are being produced.

## Summary

Five subjects of varying degrees of fitness ran for two or three different durations. In the most unfit subject 30 min of running was sufficient to produce post-exercise ketosis but the fitter subjects required a longer period of running.

## ii The effect of a second period of running

Courtice and Douglas (1936) found that a second period of exercise produced no change in the level of ketone bodies in the blood, and Heilson (1947) observed that a second period of exercise caused a decrease in the blood level. Drury, Wick and Mackay (1941) found that a short bout of heavy exercise caused a drop in the ketonaemia of rats which were already in a state of ketosis and noted similar changes in man after heavy exercise, though not after light exercise. In this section the effect of a second period of exercise has been studied.

### Methods

Three untrained subjects ran for two periods of 30 minutes and blood samples were analysed for pyruvate, acetoacetate, 3-hydroxybutyrate, glucose and FFA. On another occasion two of these subjects ran for one 30 min period under similar conditions (no food for at least 4 hr previously).

### Results

The second period of exercise was accompanied by a drop in the level of ketone bodies, whereas no decrease

took place over the same time without the second period of exercise (Fig. 5:3). In one subject the two exercise periods were separated by 2 hr instead of 1 hr but the rise in ketone bodies after the first period of exercise was very small and the decrease observed during the second period of exercise was not pronounced. Each period of running was marked by raised levels of pyruvate but there was no fall in the raised level of plasma FFA during the second run and glucose concentrations decreased about 5 mg% in each run.

### Discussion

The higher levels of ketonaemia found immediately after a run of  $1\frac{1}{2}$  hr compared to those levels found after the 30 min run (described in the first part of this chapter) suggested that in the extra hour of exercise ketone bodies if they were being used as fuel at all, were not being used at a rate equal to their production. The decrease in levels observed during a second period of exercise indicates that muscular activity at least accelerates their disposal in man. It is known that several tissues, notably the myocardium, are capable of using acetoacetate and 3-hydroxybutyrate as a fuel and may even use them in preference to glucose and glycolytic intermediates (Barnes et al., 1938; Williamson and Krebs, 1961) and long-chain fatty acids

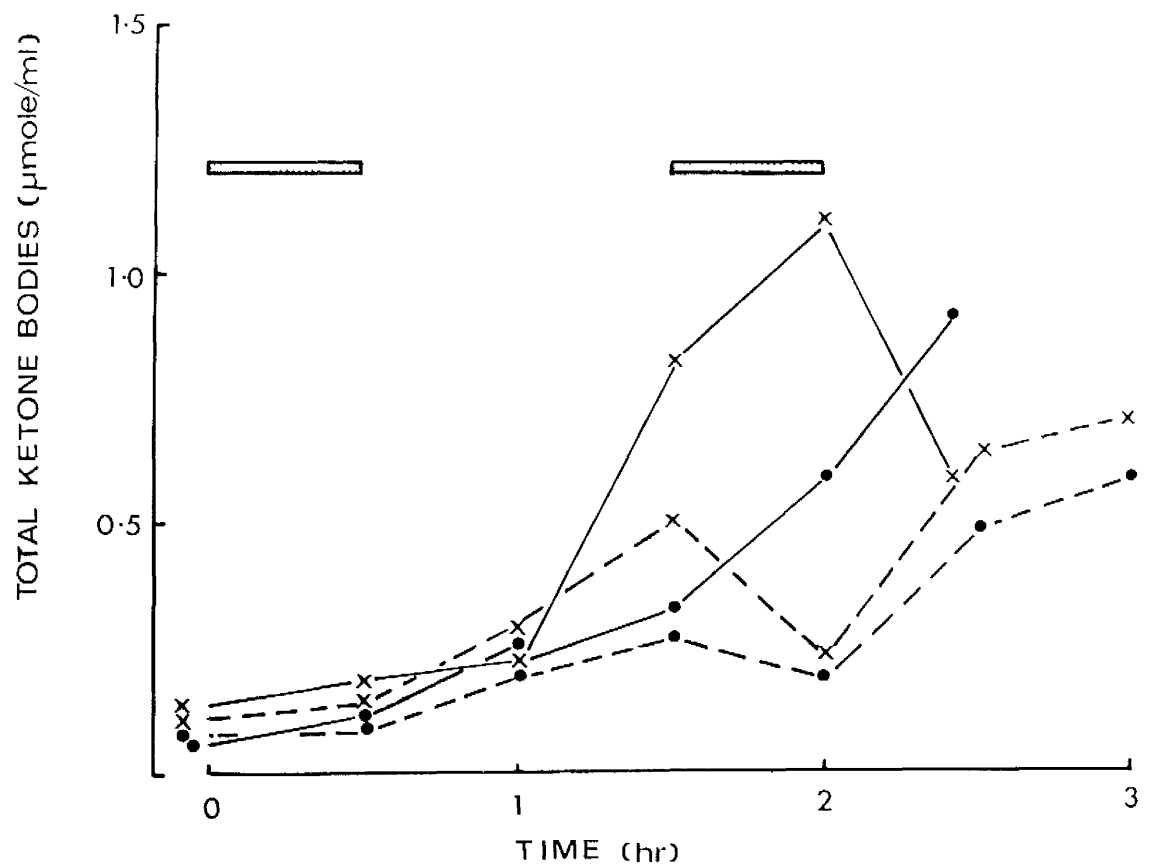


Fig. 5:3 Total blood ketone bodies ( $\mu\text{mole/ml}$ ) in two subjects after a single  $\frac{1}{2}$  hr period of running (solid lines), and after two  $\frac{1}{2}$  hr periods of running, which were separated by an hour (dashed lines). The bars represent the period of running.



(Bassenge et al., 1965). The rate of utilisation of ketone bodies is probably dependent on their blood concentration (Bates, Krebs and Williamson, 1968) rather than upon any shortage of other metabolites and in these observations at least there appeared to be no such shortage.

#### Summary

Three untrained subjects ran for two 30 min periods with an hour of rest in between. The second period of exercise was accompanied by a marked transient reduction in the level of blood ketone bodies. No such reduction occurred after a single run of 30 min.

## CHAPTER 6

### Glucose metabolism and its effect on post-exercise ketosis

The concentration of ketone bodies in the blood rises after severe exercise in untrained subjects (Chapter 3). This post-exercise ketosis has been shown to be diminished by oral administration of glucose (Courtice and Douglas, 1936; Winkler and Hebeler, 1939) but the causes of the post-exercise ketosis and of the glucose effect are not clearly understood, and in order to analyse the factors which may be responsible for these phenomena the changes in the concentrations of metabolites connected with the development of ketosis, i.e. glucose, acetoacetate, 3-hydroxybutyrate, glycerol and F F A in the blood after strenuous exercise, and the effect of glucose on these changes, have been systematically investigated. Glycerol was included because it is an indicator of mobilization of adipose tissue F F A. To determine whether post-exercise ketosis is similar in origin to the ketosis of starvation the effects of glucose administration on the blood metabolites after prolonged starvation have also been investigated. The results provide a basis for an explanation of the causes of post-exercise ketosis.

## Methods

Subjects Twenty-one male medical students, aged 19 to 23, none of whom were in regular athletic training were divided into two groups: 12 received glucose after exercise (mean weight  $\pm$  S.D.  $69.8 \pm 5.3$  kg; mean height  $\pm$  S.D.  $176.7 \pm 7.4$  cm) and 9 acted as controls (mean weight  $66.6 \pm 1.9$  kg; mean height  $175.5 \pm 5.8$  cm); they received no glucose after exercise. For the study of the ketosis of fasting 3 patients undergoing a 72 hour fast for the investigation of intermittent hypoglycaemia were available. They were given glucose at the end of their period of fasting.

Procedure The running tests were carried out between 11 a.m. and 8 p.m.; all started about 3 hours after the previous meal. The subjects ran for 90 minutes and covered distances from 14 to 18 km. Blood specimens were taken before exercise, at the end of exercise and at 30 minute intervals for 2 to 3 hours thereafter. Glucose (50 g in 100 ml water) was given orally 30-60 min after the end of the exercise. The treatment of blood samples and the analytical methods have been described in the section on techniques.

## Results

### Exercised subjects not given glucose

Blood glucose in these subjects remained steady at values slightly lower than resting (pre-exercise) levels (Fig. 6: 1 ). Blood ketone bodies (acetoacetate plus hydroxybutyrate) and plasma free fatty acids continued to rise for up to 2 hrs after the end of exercise, the increase in fatty acid concentrations above resting levels being less than those of ketone bodies (Figs. 6: 2 and 6: 3). Blood glycerol fell after exercise and then remained at a plateau considerably above resting levels (Fig. 6: 4).

### Exercised subjects given glucose

Blood glucose concentrations rose from levels slightly below those at rest to a mean 152.5 mg % 30 min after glucose and a mean of 157 mg % after 60 min (Fig. 6: 1). The hyperglycaemia was accompanied by a temporary fall in the concentrations of ketone bodies, F F A and glycerol (Fig. 6: 2, 6: 3 and 6: 4). At the nadir the concentrations of ketone bodies did not reach the resting levels whilst those of the free fatty acids and glycerol did. The concentrations of all three metabolites rose again during the second hour after glucose ingestion (Fig. 6: 2, 6: 3 and 6: 4).

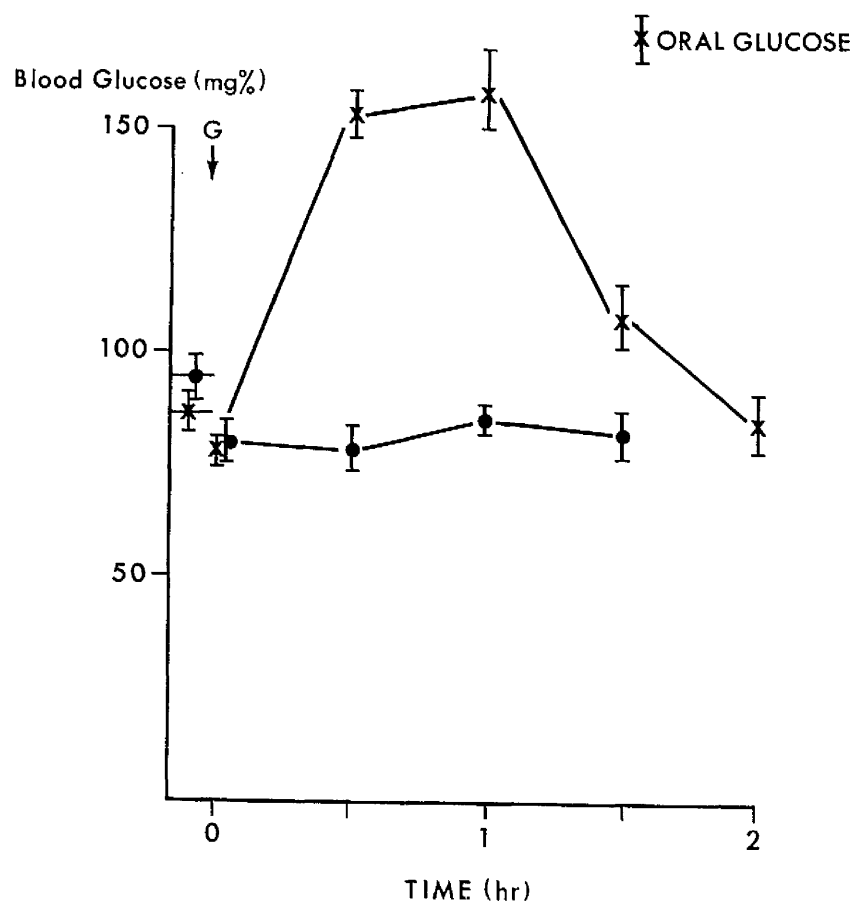


Fig. 6:1 Blood glucose (mean  $\pm$  1 S.E.M.) in 12 subjects (x---x) given 50 g of glucose orally (G) 30 and 60 min after running for  $1\frac{1}{2}$  hr, compared with 9 otherwise similar subjects (o---o) who were not given glucose. The initial points are the resting values.

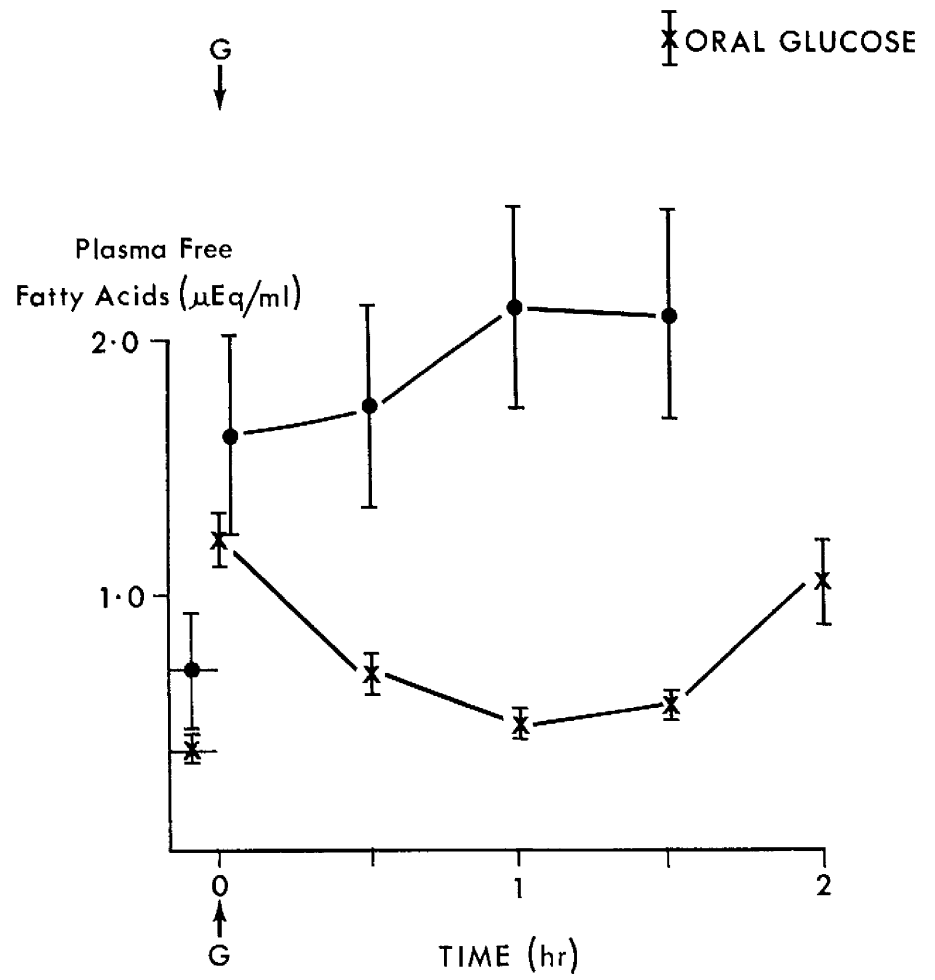


Fig. 6:2 The effect of glucose upon changes in plasma free fatty acids after exercise. The symbols are similar to those of Fig. 6:1.

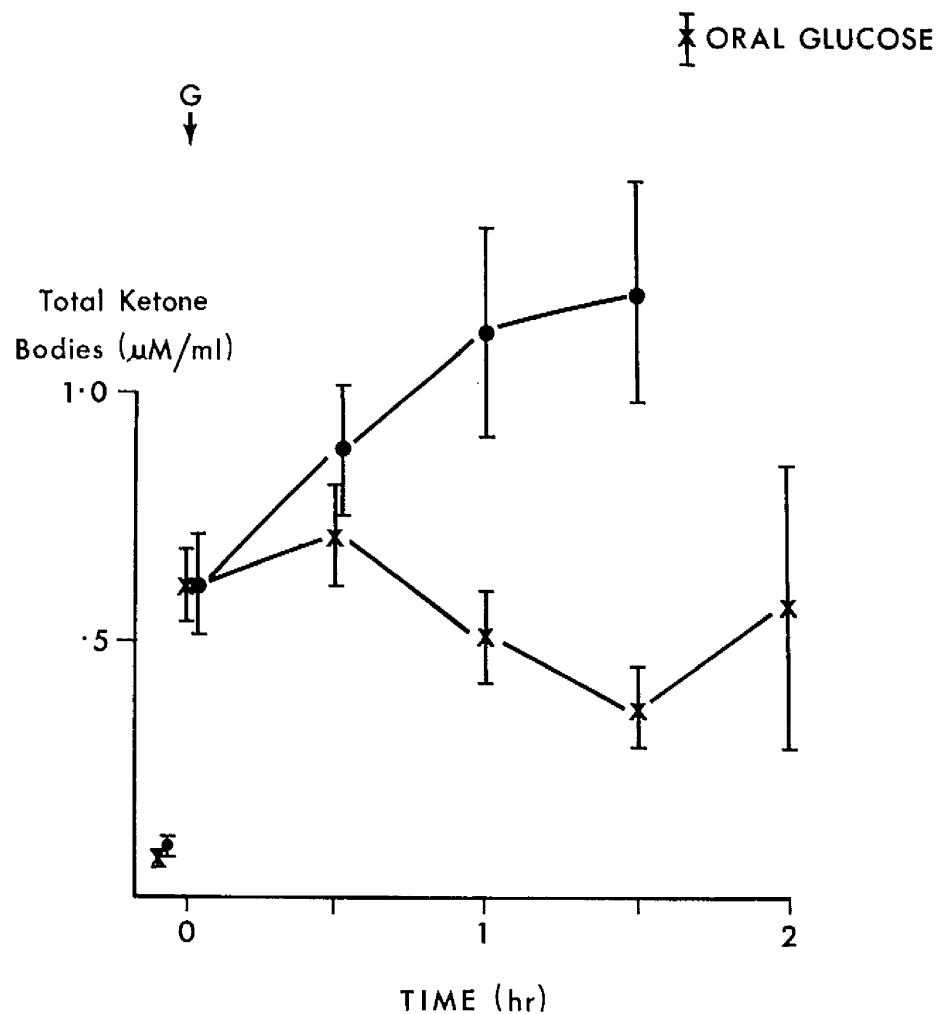


Fig. 6:3 The effect of oral glucose upon changes in to  
 levels of blood ketone bodies (3-hydroxybutyr  
 plus acetoacetate) after exercise. The symbol  
 are similar to those in Fig. 6:1.

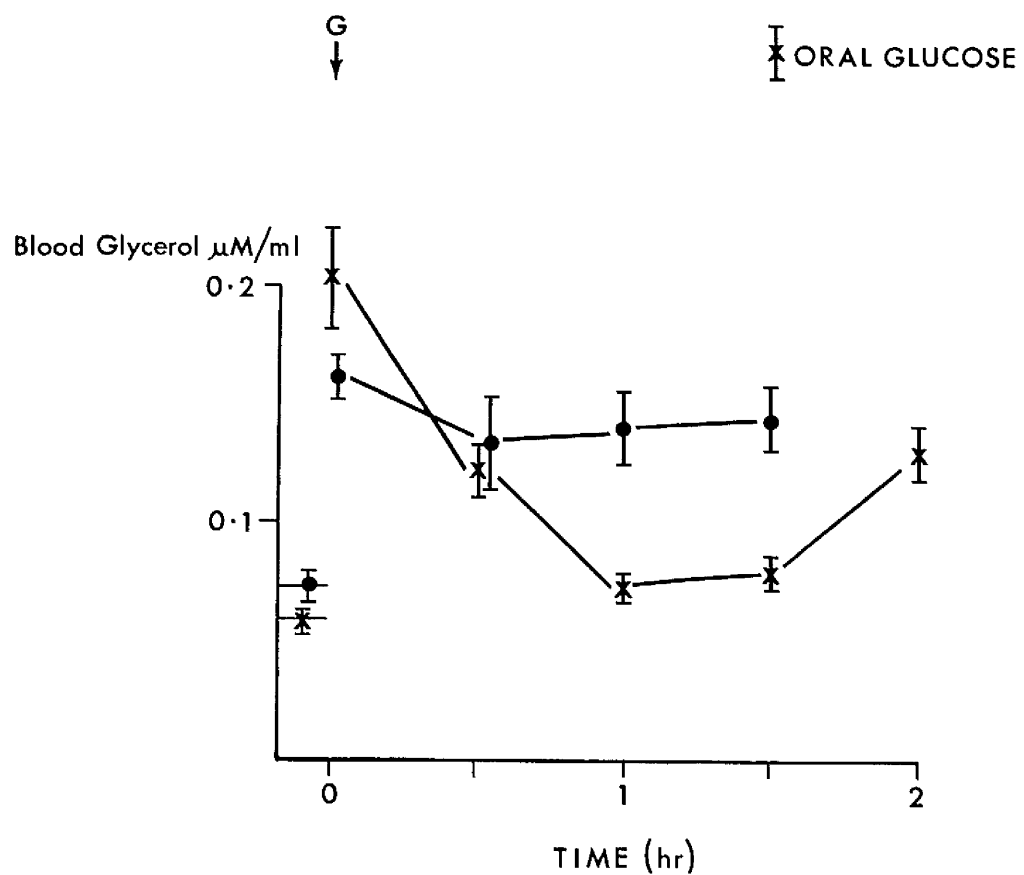


Fig. 6:4 The effect of oral glucose upon changes in glycerol levels after exercise. The symbols are similar to those of Fig. 6:1.



### Glucose tolerance tests at rest

Ingestion of 50 g of glucose by 8 of these 12 subjects in a resting state after an overnight fast (16 hours) gave normal glucose tolerance curves with peak values in all cases at 30 min after glucose (Fig. 6: 5). Blood glucose levels returned to initial values sooner at rest than after exercise. Administration of glucose caused a decrease in the concentration of ketone bodies and plasma free fatty acids, but the initial concentrations and the absolute changes were considerably less than those after exercise (Figs. 6:6 and 6:7). The extent and rate of these depressions before and after exercise as a percentage of the initial levels have been plotted in Fig. 6: 8 and 6: 9.

### Glucose tolerance after prolonged fasting

Subjects fasted for 72 hr showed the same type of modified glucose tolerance curve as the exercised subjects, with high maximum values and a failure to return to the fasting levels within 2 hr (Table 6: 1). Plasma F F A and glycerol fell and this decrease was accompanied by a rapid and sustained fall in the concentration of ketone bodies and a decrease in the 3-hydroxybutyrate/acetoacetate ratio (Table 6:1).

Table 6:1 Concentrations of blood metabolites in fasted (72 hr) subjects after

ingestion of 50 g glucose at rest

Time	0 min	30 min	60 min	90 min	120 min
Glucose	47 $\pm$ 5.5	123 $\pm$ 27	156 $\pm$ 40	172 $\pm$ 31	189 $\pm$ 24
Free fatty acids	1.49 $\pm$ 0.89	0.79 $\pm$ 0.12	0.53 $\pm$ 0.13	0.45 $\pm$ 0.22	0.58 $\pm$ 0.29
Glycerol	0.16 $\pm$ 0.07	0.13 $\pm$ 0.06	0.11 $\pm$ 0.05	0.08 $\pm$ 0.03	0.10 $\pm$ 0.01
3-Hydroxybutyrate	2.25 $\pm$ 0.26	1.57 $\pm$ 0.66	0.91 $\pm$ 0.53	0.48 $\pm$ 0.24	0.29 $\pm$ 0.20
Acetoacetate	0.63 $\pm$ 0.34	0.49 $\pm$ 0.32	0.41 $\pm$ 0.33	0.28 $\pm$ 0.17	0.11 $\pm$ 0.07
<u>3-Hydroxybutyrate</u> Acetoacetate	3.6	3.4	2.2	1.7	2.6
Ketone bodies	2.88 $\pm$ 0.61	2.06 $\pm$ 0.98	1.32 $\pm$ 0.86	0.76 $\pm$ 0.38	0.39 $\pm$ 0.34

The results are means and standard deviations of three subjects. The glucose values are mg% whole blood; other metabolites are expressed in  $\mu$ mole/ml whole blood and free fatty acids in  $\mu$ equiv/ml plasma.

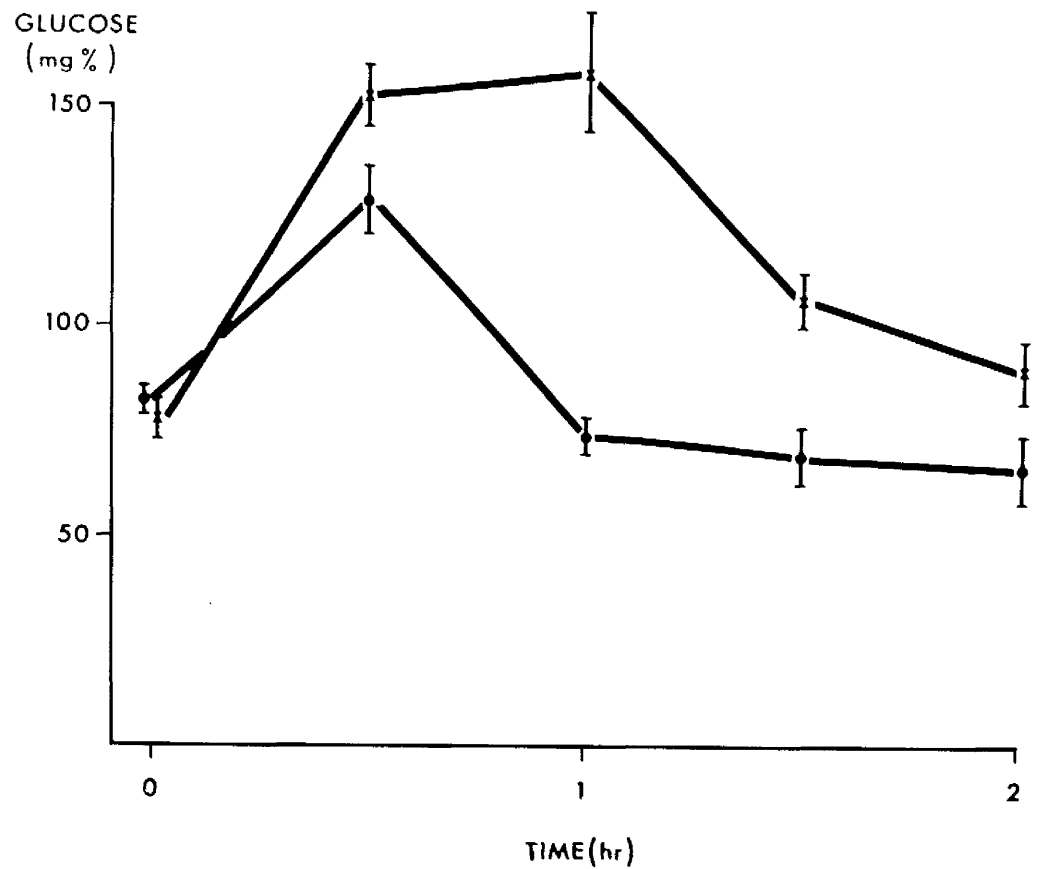


Fig. 6:5 Blood glucose in subjects given 50 g of glucose orally, at rest (○---○) and after running (●---●). The results are expressed as means  $\pm 1$  S.E.M.

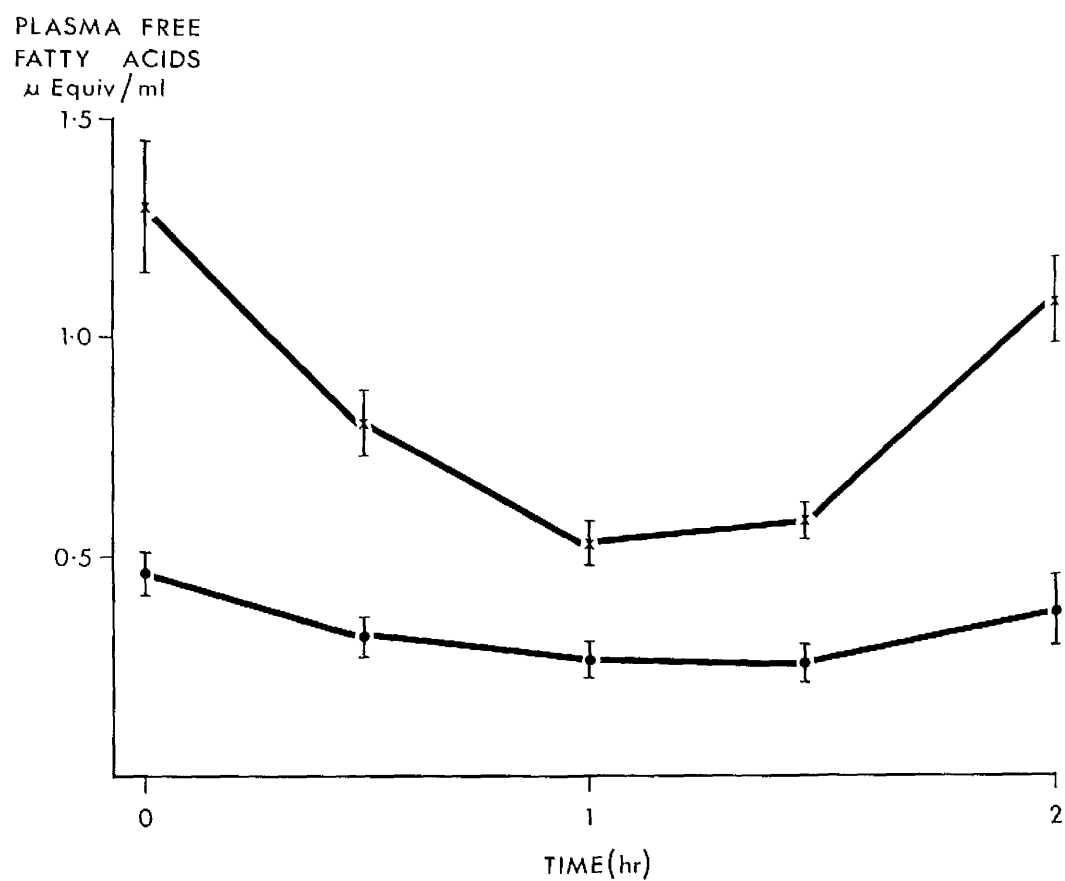


Fig. 6:6 Changes in plasma free fatty acid levels before (o---o) and after exercise (x---x) in response to a glucose (50 g) load. The results are expressed as means  $\pm 1$  S.E.M.

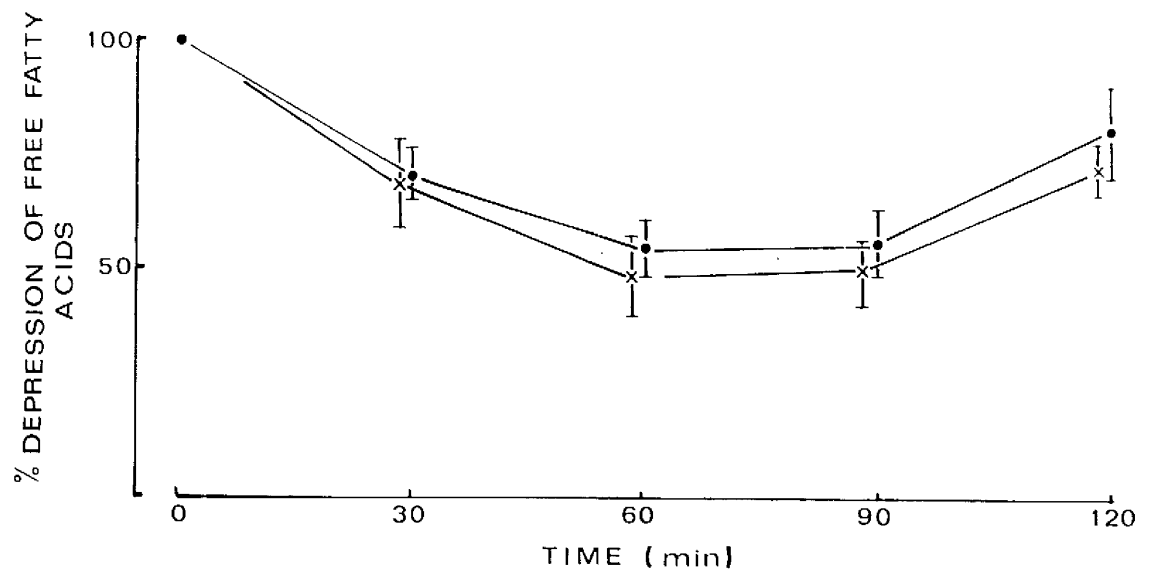


Fig. 6:7 The changes in free fatty acid (FFA) levels shown in Fig. 6:6 expressed as percentage changes of the FFA level when glucose was given.

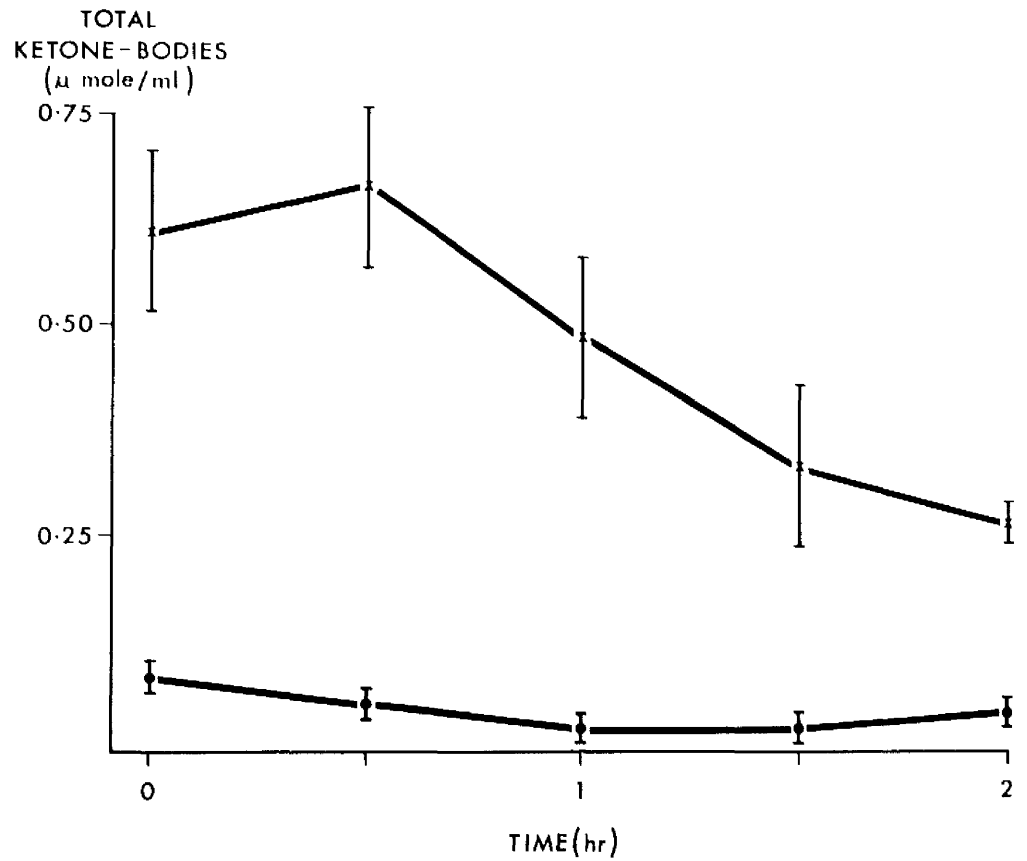


Fig. 6:8 Changes in blood ketone bodies before and after exercise in response to a glucose load. The symbols are as in Fig. 6:5.

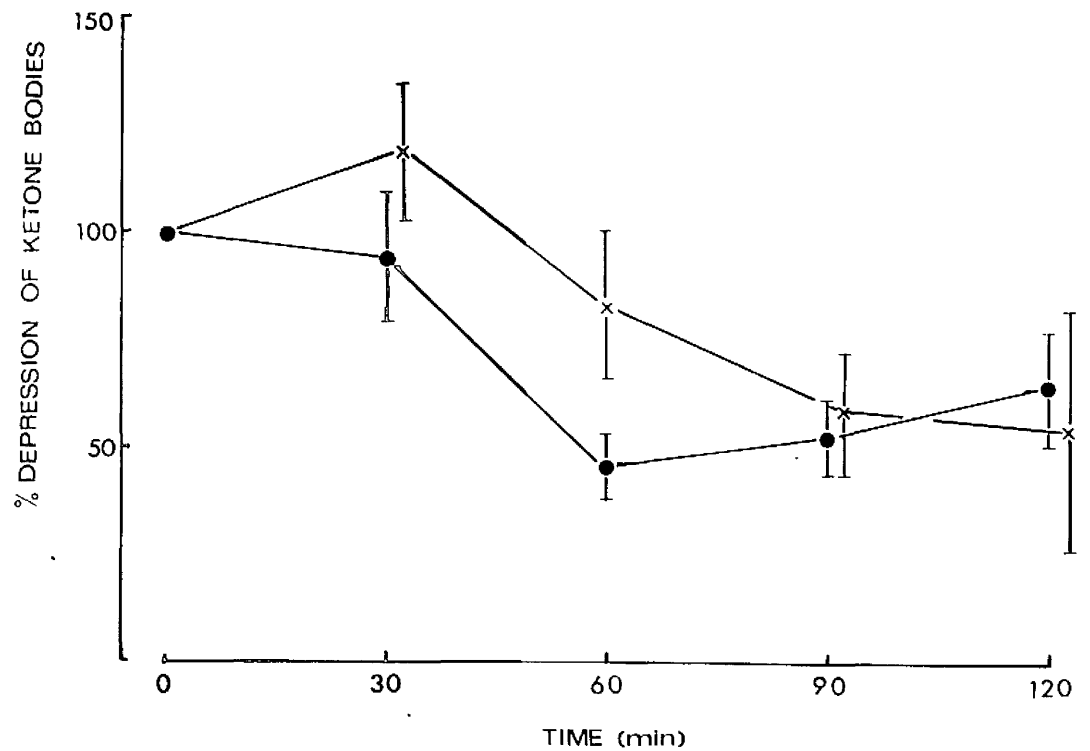


Fig. 6:9 The changes in blood ketone bodies shown in Fig. 6:8 expressed as percentage changes of the ketone body level when glucose was given. The symbols are as in Fig. 6:5.

## Discussion

### Origins of post-exercise ketosis

The results of the present and previous experiments described in Chapter 3 suggest the following explanation for the phenomenon of post-exercise ketosis. During prolonged exercise F F A are mobilized from adipose tissue and their concentration in the blood plasma rises (Carlson and Ekelund, 1963; Havel, 1965). This serves to provide a fuel of respiration for the muscles. As the concentration of plasma F F A rises the rate of formation of ketone bodies also rises. This relationship has been established for many situations such as fasting (Cahillet al., 1966), adrenaline infusion (Willms et al., 1969) and after exercise (Chapter 3). Approximate proportionality between the concentration of F F A and the rate of ketone body formation has also been demonstrated in perfused livers of starved rats (Krebs et al., 1969). The rise in the rate of ketone body formation during exercise however does not result in a major rise in the concentrations of ketone bodies in blood because the rate of utilization of ketone bodies also increases (Drury, Wick and Mackay, 1941; Blixenkrone - Møller, 1969). When exercise ceases the increased rate of ketone body formation continues because the plasma concentrations of F F A remain elevated and this, together with a



decreased rate of ketone body utilization after cessation of exercise, would cause the post-exercise ketosis. Passmore and Johnson (1958) have reached similar conclusions concerning the changes of ketone body concentrations during and after exercise.

There is, in addition to the plasma concentration of F F A, another major factor that determines the rate of ketone body production by the liver. This is the availability of carbohydrate. In the present experiments administration of glucose rapidly diminishes the ketosis of starvation and post-exercise ketosis. This is probably due partly to the fall in plasma F F and partly to a decreased ketone body formation from F F A in the liver (Wieland and Matschinsky, 1962; Williamson et al., 1969). Thus the degree of post-exercise ketosis is liable to depend to some extent on the amount of hepatic glycogen, as indicated by negligible post-exercise ketosis in subjects previously fed on a high carbohydrate diet (Courtice and Douglas,

Ketosis of starvation and post-exercise ketosis thus share as a common feature the shortage of carbohydrate to meet energy needs and the replacement of this fuel by F F A.

Effect of exercise on the blood glucose tolerance curve

An 'abnormal' glucose tolerance curve after strenuous exercise was first observed by Courtice, Douglas and Priestley (1939). The shape of the curve after exercise is very similar to that after prolonged fasting or in subjects given a low carbohydrate diet (Cahill et al., 1965; Unger, Eisentraut and Madison, 1963; Rooth, Kagedal and Carlstrom, 1969). This modification has been attributed to a diminished insulin secretion but measurements of plasma insulin have not confirmed a major deficiency in the insulin output (Unger, Eisentraut and Madison, 1963; Hales and Randle, 1963; Gemuth, 1966) although there has been a slightly delayed response. The modification of the glucose tolerance curve is more likely due to an inhibition of glucose utilisation by the high concentration of fatty acids and ketone bodies. Such an inhibition has in fact been demonstrated in striated muscle by Garland, Newsholme and Randle (1962) and Williamson and Krebs (1961).

### Summary

The effects of ingestion of glucose after strenuous exercise on the concentrations of blood glucose, glycerol, ketone bodies and plasma free fatty acids have been studied in a group of twelve subjects.

The resulting hyperglycemia was accompanied by decreases in the concentrations of glycerol, F F A and ketone bodies. Comparison of these results with those of oral glucose tolerance tests at rest in eight of the same subjects indicated reduced glucose tolerance after exercise. A similar pattern of metabolite changes was observed on administration of glucose to three subjects undergoing a prolonged fast (72 hr ). The findings support the hypothesis that post-exercise ketosis is due to elevated F F A concentrations and depletion of carbohydrate stores.

## CHAPTER 7

### Acetoacetate Tolerance

In Chapter 3 it was shown that well-trained athletes are capable of maintaining low levels of blood ketone bodies during and after strenuous exercise. In subjects not in athletic training the blood concentrations increase after exercise due either to a higher rate of production of ketone bodies or to a lower rate of utilisation. If the rate of utilisation is different in the two groups, then the tolerance curves after ingestion of acetoacetate should be different. To investigate this problem the tolerance to oral acetoacetate before, during and after exercise in a group of trained athletes has been compared with a similar series of investigations upon untrained subjects. The development of ketosis after exercise has also been studied in these subjects.

In an appendix to this chapter, a study of the secretion of growth hormone during acetoacetate tolerance tests in four subjects is reported.

### Methods

#### Tolerance to acetoacetate at rest

Subjects 13 male subjects (aged 19-34 yr) were studied. Their participation in athletic training ranged from nil to running 120 km/week.

Procedure Sodium acetoacetate (200 ml; 0.4 M) (Krebs

and Eggleston, 1945) was taken orally after a fast of at least 6 hr. The subjects remained at rest throughout the investigation. Venous blood samples were taken beforehand after 40 min and then at subsequent 20 min intervals for a further hour. The blood samples were analysed as described in Chapter 1 for acetoacetate, 3-hydroxybutyrate, glucose, lactate, pyruvate, glycerol and plasma free fatty acids.

The subjects were also studied after a period of running of  $\frac{1}{2}$ -1 $\frac{1}{2}$  hr to determine whether or not post-exercise ketosis developed.

#### Acetoacetate tolerance during and after exercise

Subjects 9 subjects (3 trained athletes and 6 untrained subjects) were further investigated during and after running.

Procedure Sodium acetoacetate (200ml; 0.4 M) was given orally immediately before and 30 min after a 1 $\frac{1}{2}$  hr period of running. Venous blood samples were taken before ingestion of acetoacetate, 40 min later and on three further occasions at approximately 20 min intervals until the end of the exercise. A sample was taken 30 min later just before the ingestion of the second dose of acetoacetate 40 min later, and then at three subsequent 20 min intervals. The samples were analysed as before.

## Results

### Tolerance to acetoacetate ingestion at rest

The ingestion of this quantity of sodium acetoacetate produced a peak level of total blood ketone bodies of between 0.4 and  $1.7\mu\text{mole/ml}$  40 minutes after ingestion. Five of the subjects were in athletic training and did not show raised levels of ketone bodies after exercise whereas the others did (Fig. 7:1). The tolerance curves of these five subjects, though they showed a wider spread of peak levels, were similar to those of the eight subjects who developed post-exercise ketosis (Fig. 7:2).

The administration of acetoacetate was accompanied by a small drop in blood glucose and plasma free fatty acid and a more marked fall in blood glycerol levels. There was no difference between the athletic and the non-athletic subjects. The magnitudes of these changes are shown for 4 subjects in Figs. 7:3, 4 and 5.

### Acetoacetate tolerance during and after exercise

The nine subjects showed considerable differences in their blood levels of ketone bodies after exercise. The three athletes showed no ketosis whereas the other six subjects showed a wide range of post-exercise ketosis ( $0.6 - 1.4\mu\text{mole/ml}$  at 1 hr). Both groups showed an increased tolerance to acetoacetate during exercise as compared to their tolerance curves at rest (Fig. 7:6). After exercise

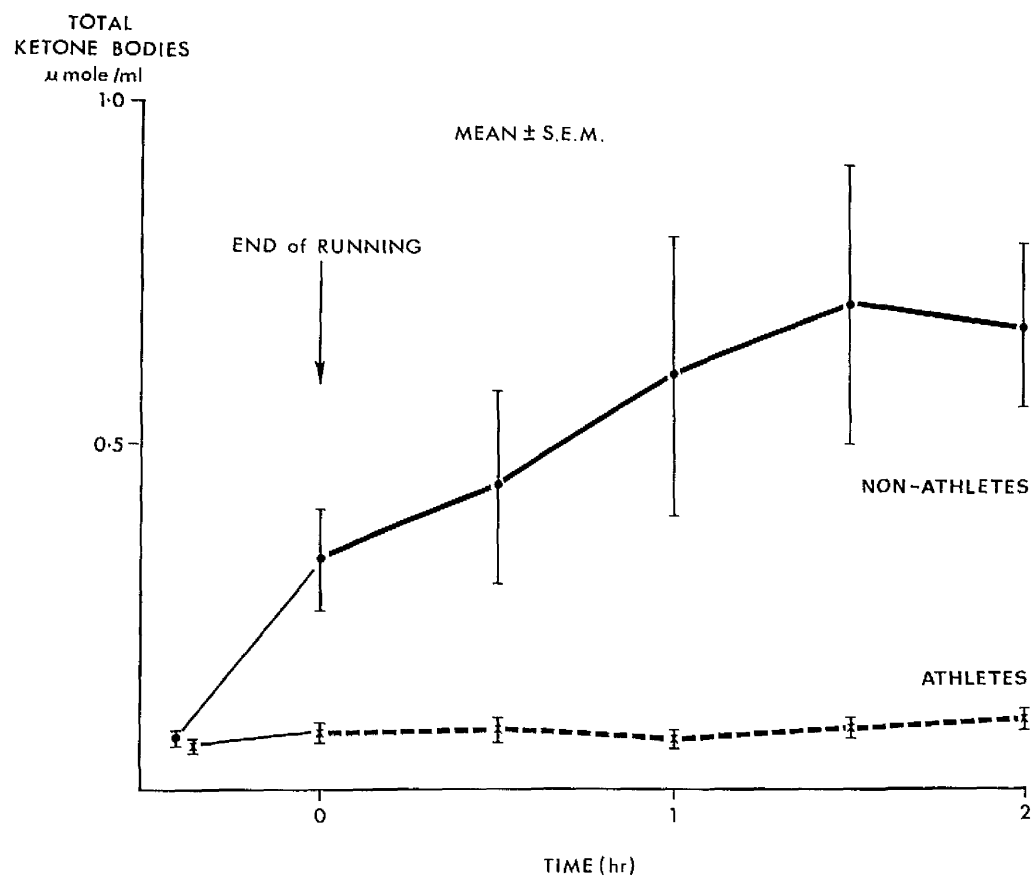


Fig. 7:1 Total ketone bodies in 8 non-athletes and 5 trained athletes before and after running.

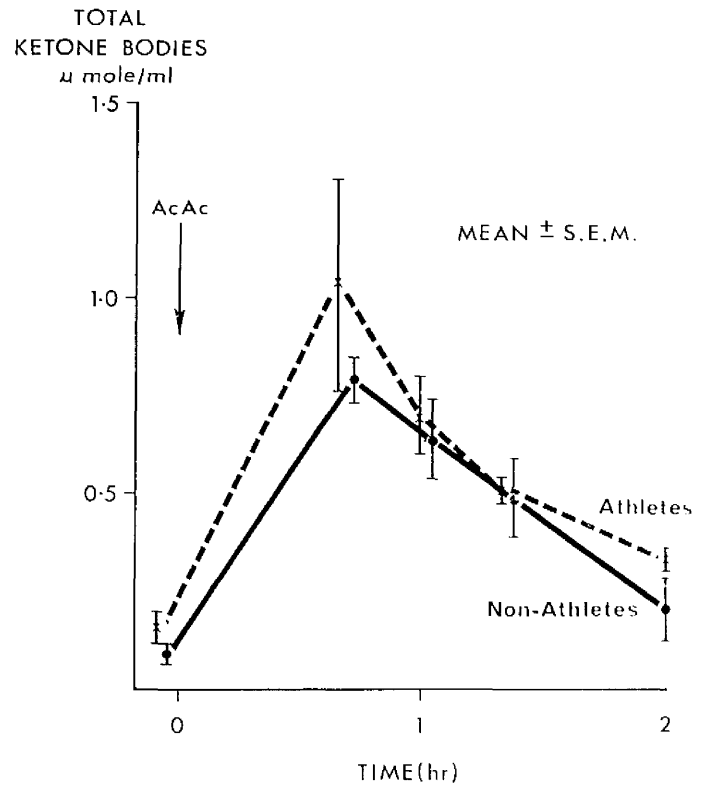


Fig. 7:2 Total ketone bodies in 3 non-athletic subjects (o—o) and 5 trained athletes (x—x) after the ingestion of sodium acetoacetate ( 200mg 0.4 M ).



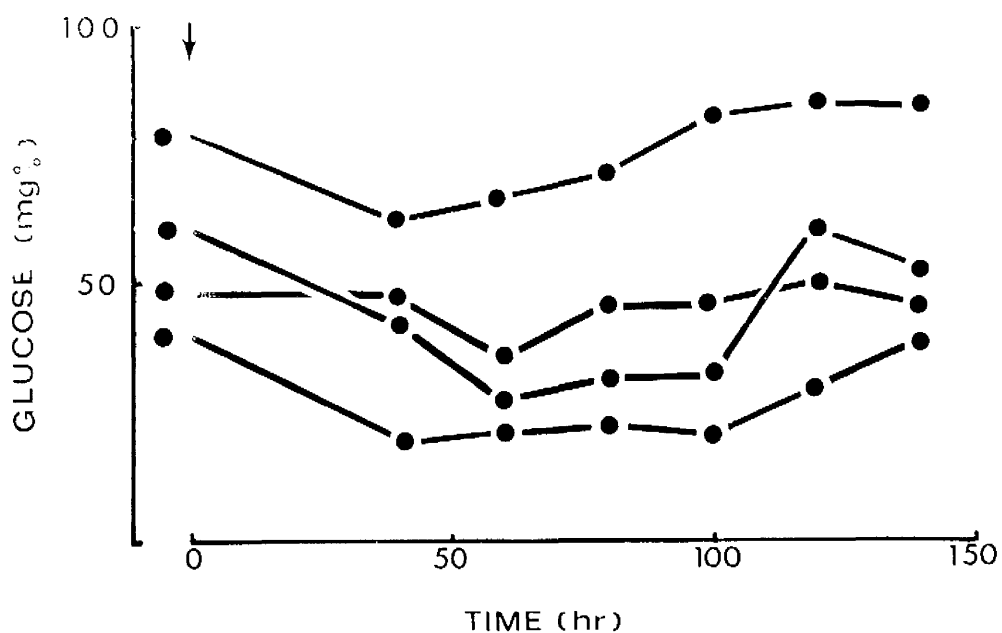
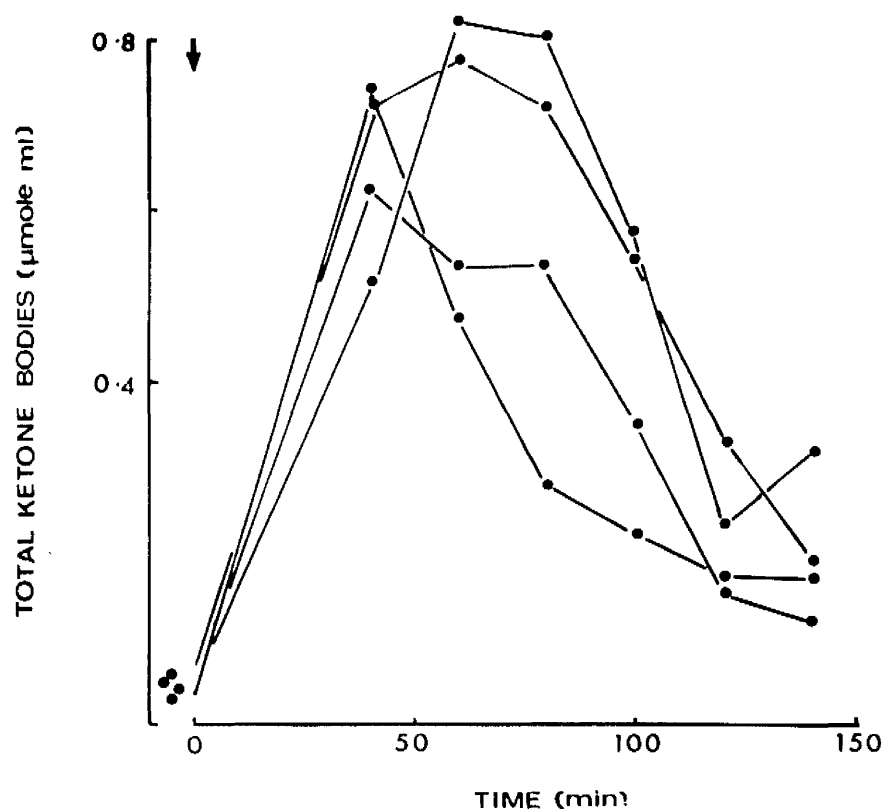


Fig. 7:3 Total ketone bodies (above) and glucose (below) after ingestion of acetoacetate in the same four subjects.

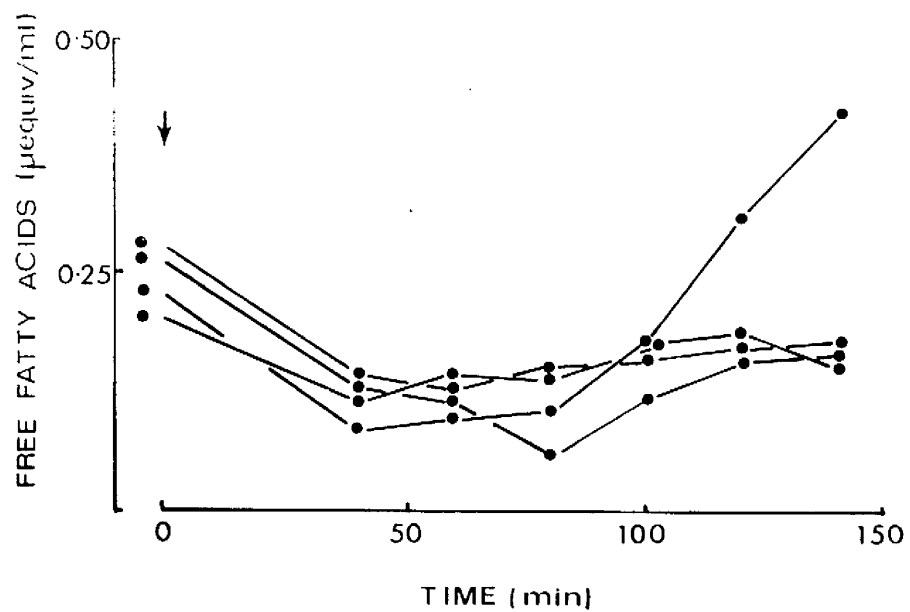


Fig. 7:4 Plasma free fatty acids after the ingestion of acetoacetate in the same subjects as in fig. 7:3.

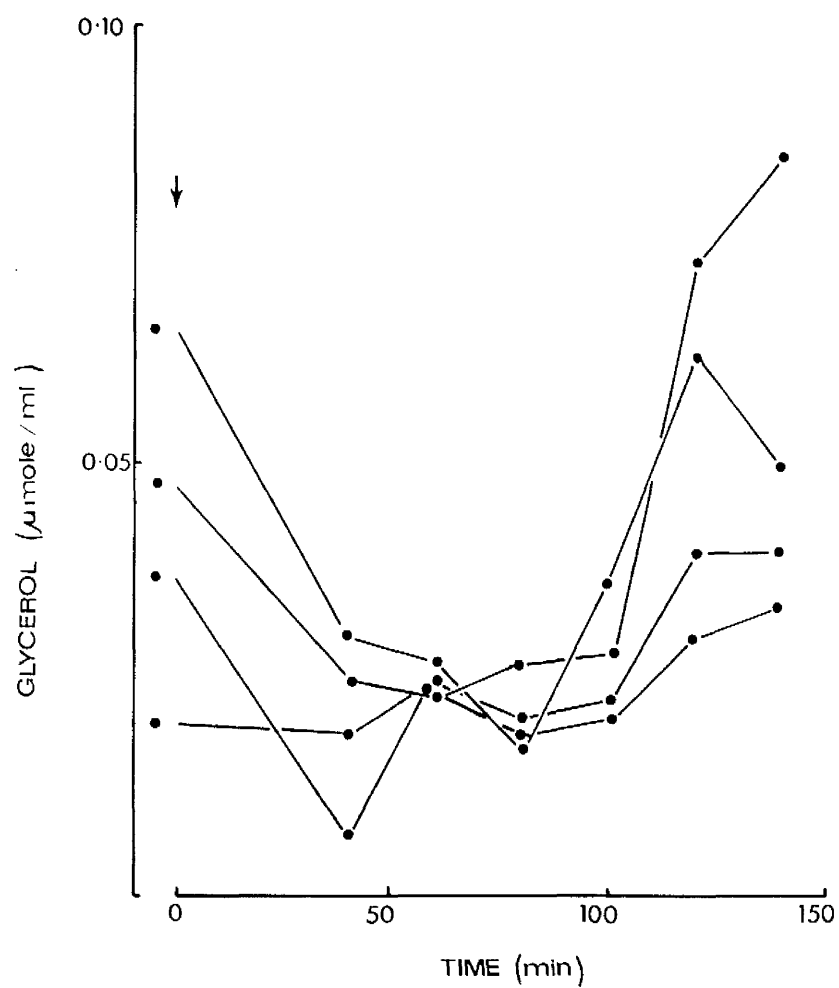


Fig. 7:5 Blood glycerol after the ingestion of acetoacetate in the four subjects shown in fig. 7:3.

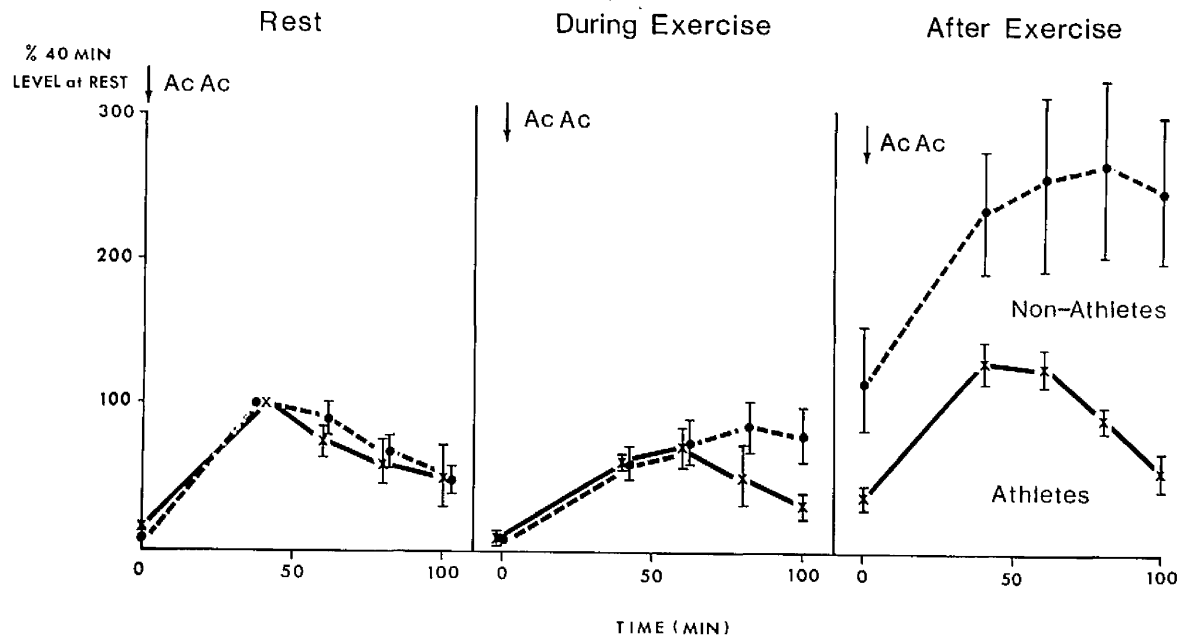


Fig. 7: Acetoacetate tolerance curves in 3 trained athletes (x) and 6 untrained subjects (•) before, during and after exercise. The results are expressed as percentages (mean  $\pm$  1 S.E.M.) of the level of total ketone bodies 40 min after acetoacetate was ingested at rest.

6

tolerance was decreased in both groups but the peak levels of total ketone bodies reached in the athletes was 130% of the 40 minute level in the curve when the subject was resting, while in the untrained group the corresponding value was 240%. Subtraction of the values obtained after exercise alone indicated that the peak level reached in the athletes was similar to the corresponding value for the ingestion at rest while in the untrained subjects the peak level was still raised (Fig. 7:7).

Glucose levels decreased after the ingestion of acetoacetate and this was more marked after the post-exercise dose of acetoacetate. This depression was greater in the untrained group (Fig. 7:8).

Blood glycerol increased by similar amounts (to  $0.25 - 0.30 \mu\text{mole/ml}$ ) in both groups of subjects towards the end of the exercise period and then decreased. Plasma free fatty acids also increased towards the end of exercise reaching higher values in the untrained group compared with the athletic group (Fig. 7:9).

### Discussion

These investigations were carried out to examine the mechanisms whereby athletes do not develop post-exercise ketosis as readily as untrained subjects. At rest no marked difference was found in the rate of utilisation of acetoacetate between the unfit subjects who developed

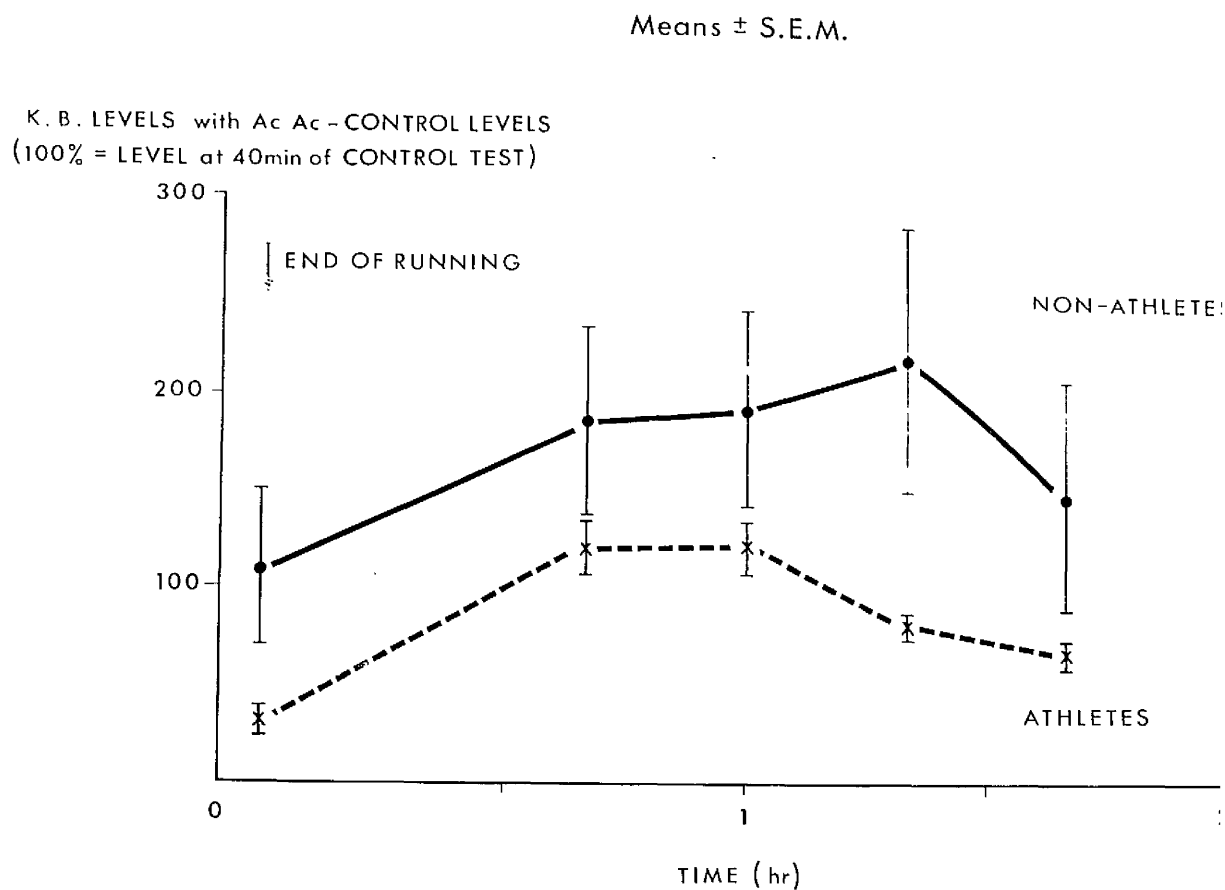


Fig. 7:7 Total blood ketone bodies in the subjects of fig. 7:6 when acetoacetate was ingested after running. The levels of ketone bodies obtained after exercise alone have been subtracted from the actual levels of fig 7:6 and the results expressed as percentages of the peak level after acetoacetate ingestion at rest.

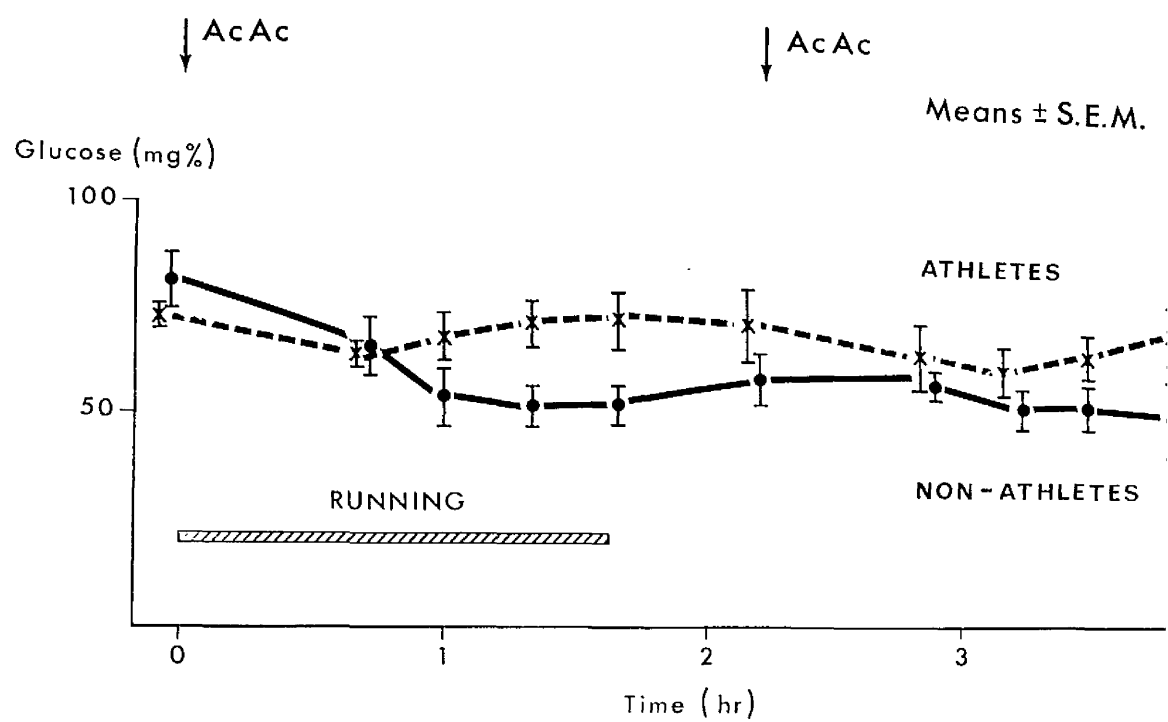


Fig. 7:8 Blood glucose levels during and after exercise with the ingestion of acetoacetate.

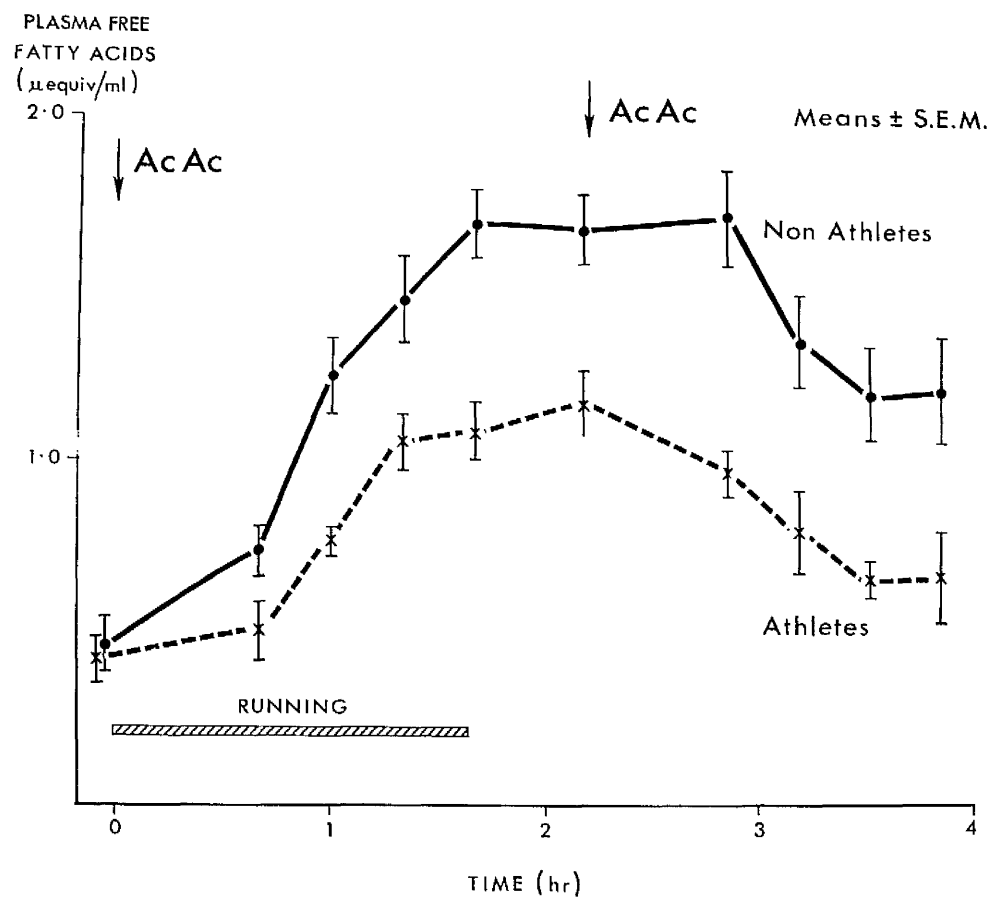


Fig. 7:9 Plasma free fatty acids during and after exercise with the ingestion of acetoacetate.



post-exercise ketosis and the athletes who did not.

Therefore, whether or not ketosis develops probably depend on differences in the rates of ketone production. A similar situation appears to exist in obese and non-obese subjects. Kekwick, Pawan and Chambers (1959) showed that though obese people had a higher resistance to ketosis, their rate of metabolism of infused hydroxybutyrate was similar to that observed in the non-obese.

The increased tolerance to acetoacetate during exercise suggests that ketone bodies are being utilised as a fuel. Such a role for ketone bodies has been demonstrated previously both in the whole animal (sheep) (Bergman, Kon and Katz, 1963) and in isolated tissues (Krebs and Williamson, 1961) and in this instance it would explain why in Chapter 3 no marked rise in ketone body levels was found to occur during the exercise period itself. It is unlikely that these results are an expression of a reduced rate of absorption during the exercise as Fordtran and Saltin (1967) have shown that the intestinal absorption of small molecules such as glucose appears to be unaffected by exercise.

After exercise the untrained group had a much reduced tolerance to acetoacetate compared to their tolerance at rest and to the trained group. The raised level of plasma FFA suggests that the production of ketone bodies continues after exercise in the untrained group and the

probable explanation of their post-exercise ketosis would be a reduction in the rate of utilisation of ketone bodies as exercise ceases.

The trained group showed no reduction in tolerance to acetoacetate after exercise and a smaller rise in plasma FFA levels. Their ability not to develop post-exercise ketosis may be explained by a smaller production of ketone bodies and a better balance between utilisation and production after exercise.

A hypoglycaemic action of ketone bodies has been reported by Felts, Crofford and Parks (1964) and by Balasse, Couturier and Ooms (1967) who infused 3-hydroxybutyrate into dogs, and in man by Balasse and Ooms (1968). Oral acetoacetate has also been shown to depress glucose and FFA levels in man (Jenkins, 1967). The hypoglycaemic action in the intact animal appears to be mediated by stimulation of the pancreas to secrete insulin (Felts, Crofford and Parks, 1964) though the precise mechanism remains unknown. The results reported in the appendix support their observation of an increased level of insulin after acetoacetate ingestion.

The depression of glucose during and after exercise when acetoacetate was ingested was more marked in the untrained subjects. If the utilisation of ketones is seen as a glucose sparing mechanism then it would appear that

Time (min)	Ketone bodies ( $\mu$ mole/ml)	Glucose (mg%)	FFA ( $\mu$ equiv/ml)	HGH (ng/ml)	Insulin ( $\mu$ U/ml)
0	0.08	84	0.43	< 2	37
40	.64	70	0.40	"	74
60	.56	68	0.34	"	50
80	.42	69	0.35	"	47
100	.34	74	0.40	"	43
120	.37	72	0.46	"	25
180	.20	76	0.43	"	25
240	.19	73	0.45	"	28
300	.15	76	0.46	"	28

Table 7:1 Metabolite and hormonal changes after ingestion  
of acetoacetate in a healthy, fasted male  
subject.

the training an athlete carries out tends to make this mechanism more effective.

#### Appendix

The changes in the secretion of growth hormone and insulin after ingestion of acetoacetate have been followed in four subjects. One of these subjects was a healthy male ( age 19 yr ) and the other 3 were obese females ( age 45-55 yr ), one of whom was on a low calorie diet of 600 kcal per day. Growth hormone was measured by the method of Hunter and Greenwood (1962) and insulin by the method of Yalow and Berson (1960).

Ingestion of acetoacetate by the healthy male subject was accompanied by a depression of glucose and plasma FFA, a rise in plasma insulin, but no secretion of growth hormone (table 7:1). The obese subjects showed smaller rises in insulin and again no secretion of growth hormone. The obese subject on a low calorie diet had a reduced tolerance to acetoacetate and higher basal levels of ketone bodies.

These results indicate that the experimental ketosis is not associated with increased secretion of HGH despite the hypoglycaemia which can per se cause HGH secretion ( Roth et al., 1963 ).

## Summary

Tolerance to sodium acetoacetate (200ml, 0. taken orally has been studied in 13 male subjects at and concomitant changes in 3-hydroxybutyrate, glucose, glycerol and free fatty acids have been observed. The subjects ran for either  $\frac{1}{2}$  hr or  $1\frac{1}{2}$  hr on another occasion to investigate the extent to which they developed post-exercise ketosis after the run. Five subjects did not develop post-exercise ketosis, and acetoacetate tolerance curves were similar to those of the subjects who did become ketotic after running. Nine of these subjects (3 trained athletes and 6 untrained subjects) were studied further. The 3 athletes did not become ketotic after running whereas the 6 untrained subjects showed significantly raised post-exercise levels of ketone bodies. Changes in their metabolite levels were also followed when acetoacetate was ingested during and after running for  $1\frac{1}{2}$  hr. Both groups had increased tolerance to acetoacetate during exercise and decreased tolerance after exercise but the latter was more marked in the untrained subjects.

These observations suggest that the rate of metabolism of acetoacetate is similar in both groups at rest, and that acetoacetate utilisation may be increased during exercise. In the untrained subjects post-exercise ketosis probably develops because the

the rate of utilisation of ketones stops with the cessation of exercise but their production is still continuing. In the athletes production and utilisation appear to be more closely balanced.

## CHAPTER 8

### Endocrine Control

The metabolite changes reported in this thesis probably depend upon alterations in hormonal levels. Among the hormones which have been implicated in exercise metabolism is human growth hormone (HGH) and this chapter considers two problems in relation to it. The first section reports differences in HGH production between athletes and untrained subjects during exercise. The second however is a study of patients with panhypopituitarism who do not produce HGH and since normal metabolite changes were observed in these subjects, it would appear that HGH is not primarily involved in the metabolic responses considered.

#### i Differences in growth hormone production in athletes and untrained subjects

Growth hormone is known to be released in response to exercise (Hunter, Fonseka and Passmore, 1965) and in turn it increases the rate of adipose tissue lipolysis (Raben and Hollenberg, 1959). In view of the differences between athletes and untrained subjects in the development of post-exercise ketosis and the connection between lipolysis and ketosis, there might be a corresponding difference in the release of

growth hormone. Unfortunately while these experiments were being analysed a letter was produced by Sutton et al., (1968) which reported differences between athletes and untrained subjects.

### Methods

Two well-trained athletes and two untrained subjects ran for 90 min after fasting for at least 6 hr. The particulars of the subjects are given in Table 8: 1.

Blood samples were taken before the exercise, after 30 min, at the end of exercise, and at three subsequent 30 min intervals. They were analysed for lactate, pyruvate, acetoacetate, 3-hydroxybutyrate, glycerol and plasma free fatty acids (Chapter 1). The plasma was also analysed for HGH by Dr. W.P. Dugan using a modification of the method of Hunter and Greenwood (1962 and 1964).

### Results

The athletes ran faster than the untrained subjects and had much lower pulse rates during exercise (table 8: 1). The athletes also showed smaller increases in blood lactate and pyruvate concentrations during the exercise.



Subject	Age (yr)	Weight (kg)	Height (cm)	Train (km/week)	Speed (km/hr)	Heart rates min during exercise		
Trained	1	28	173	140	15.2.	116	122	132
	2	26	177	100	15.2.	136	140	146
Untrained	3	20	182	0	10.9	168	160	165
	4	19	185	0	10.0	164	168	170

Table 8:1 Details of the four subjects who took part in the investigations of Chapter 8, part i, together with their running speeds and heart rates during exercise.

Time (min)	Lactate ( $\mu$ mole/ml)		Pyruvate ( $\mu$ mole/ml)		Glycerol ( $\mu$ mole/ml)		FFA ( $\mu$ equiv/ml)		K B ( $\mu$ mole/ml)		EGH (ng/ml)	
	1	2	1	2	1	2	1	2	1	2	1	2
Rest	0.76	1.15	0.102	0.091	0.058	0.042	0.43	0.28	0.055	0.073	<1.3	7
30	1.21	1.22	0.110	0.067	0.130	0.193	0.38	0.27	0.083	0.107	20	18
90	0.72	0.81	0.064	0.067	0.398	0.484	0.48	0.61	0.153	0.119	27	26
120	0.91	0.75	0.078	0.039	0.114	0.210	0.48	0.75	0.194	0.152	13	2.5
150	0.73	0.77	0.049	0.054	0.092	0.124	0.70	0.41	0.175	0.151	1.3	<1.3
180	0.82	0.75	0.056	0.061	0.077	0.105	0.57	0.15	0.241	0.149	2.3	2.1

	3		4		3		4		3		4		3		4	
	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4
Rest	1.11	1.01	0.034	0.085	0.055	0.061	0.29	0.51	0.060	0.130	3	14				
30	5.50	6.41	0.237	0.349	0.130	0.212	0.85	0.75	0.193	0.396	>45	>30				
90	3.11	3.02	0.181	0.320	0.299	0.363	1.13	1.36	0.250	0.893	>45	>30				
120	1.60	2.17	0.101	0.241	0.179	0.227	1.35	1.44	0.412	1.260	17	22				
150	1.30	1.08	0.053	0.103	0.170	0.246	1.30	1.50	0.957	1.650	5.4	25				
180	0.88	1.00	0.058	0.079	0.178	0.180	1.18	1.38	1.464	1.860	8.0	30				

Table 8:2 Metabolite levels in the four subjects of Table 8:1, before, during and after a period of running of from 0-90 min. (K B and EGH stand for ketone bodies and Human Growth Hormone respectively).  
Subjects 1 and 2 were trained athletes, and subjects 3 and 4 were untrained.

The untrained subjects developed marked post-exercise ketosis and showed larger increases in plasma free fatty acids than the athletes. They also showed a more pronounced and sustained rise in growth hormone levels than the athletes (table 8: 2).

### Discussion

The larger and more prolonged rise in growth hormone in the untrained subjects agrees with the results obtained by Sutton et al., (1968) whose subjects exercised for 30 min on a bicycle ergometer. In Sutton's letter, however, no other metabolite measurements are mentioned.

Injection of human growth hormone into man causes a rise in plasma FFA (Raben and Hollenberg, 1959) and arterial perfusion of HGH into the fore-arm is followed by an increased FFA release from adipose tissue (Rabinowitz et al., 1965). In larger doses HGH increases tissue uptake of amino acids but it is probable that its main action is on FFA release (Hunter 1966). The more pronounced release of FFA found in the untrained subjects might then be explained by the relatively large output of growth hormone, compared to the athletic subjects. The more rapid return of HGH towards basal levels in the athletes, coupled with a decrease in ketone body utilisation as exercise ceases

might also account at least in part for the differences in ketotic response in the two groups.

ii. Metabolic changes with exercise in subjects with hypopituitarism

An opportunity of delineating the role of growth hormone in exercise, with particular reference to the post-exercise fatty acid and ketotic response is provided by studying patients with hypopituitarism

Methods

Three subjects with hypopituitarism and 3 normal untrained control subjects were studied. The particulars of the subjects are given in Table 8:4.

The subjects, who had fasted for at least 6h were exercised on a bicycle ergometer for 30 min. Blood samples (15 ml) were taken by venepuncture, before exercise, at three 10 min intervals and then at subsequent 30 min intervals for  $1\frac{1}{2}$  hr. The blood samples were treated as described in Chapter 1 and analysed for lactate, pyruvate, acetoacetate, hydroxybutyrate, glycerol and the plasma for FFA and growth hormone (see section i of this chapter).

Results

In both groups of subjects the exercise was accompanied by raised lactate and pyruvate levels (Fig. 8:1 and 8:2). Glycerol and plasma FFA increase

	Sex	Age (yr)	Height (cm)	Weight (kg)	Diagnosis
Controls					
1	M	19	170	66	-
2	M	19	184	92	-
3	M	20	135	76	-
Subjects					
1	M	61	175	78	} chromophobe adenoma and subsequent removal of the tumour
2	F	38	158	89	
3	M	17	157	49	

Table 8:4 Details of 3 controls and 3 hypopituitary subjects.

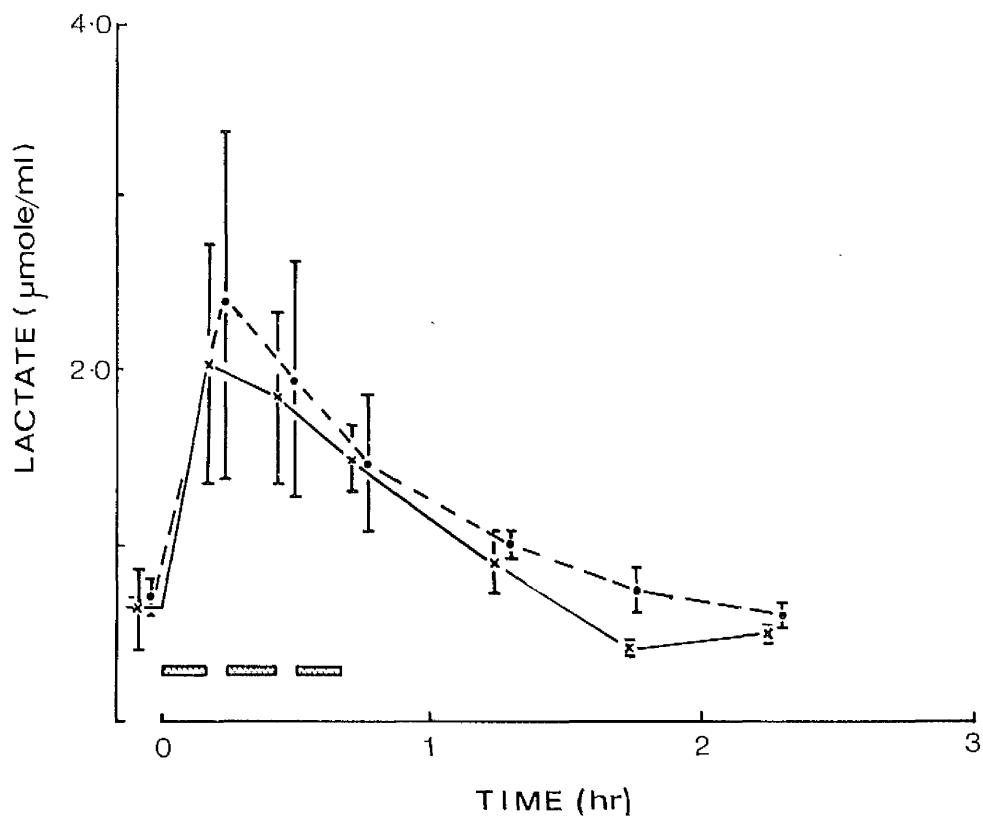


Fig. 8:1 Blood lactate levels (mean  $\pm$  1 S.D.) in 3 normal subjects (x) and 3 hypopituitary subjects (•) during and after exercise (stippled bars).

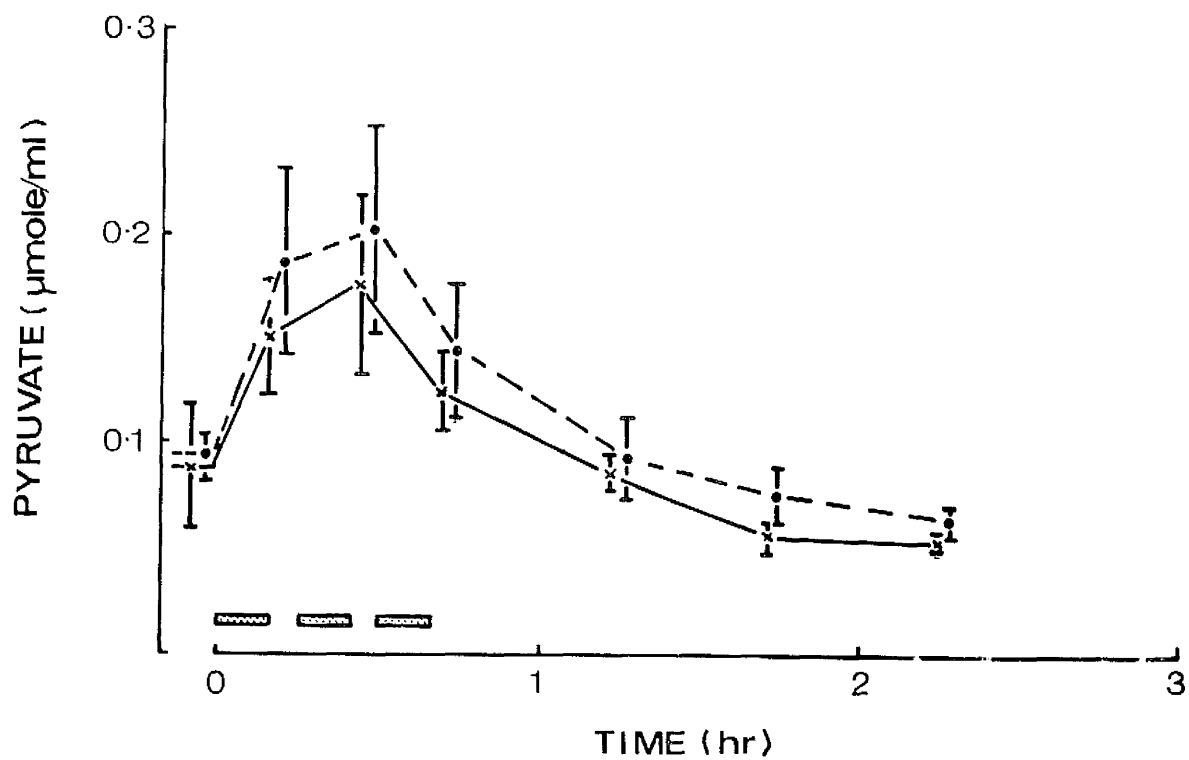


Fig. 8:2 Blood pyruvate levels (mean  $\pm$  1 S.D.) in 3 normal subjects (x) and 3 hypopituitary subjects (•) during and after exercise (stippled bars).



towards the end of exercise in all subjects, reaching higher levels in the hypopituitary subjects (FFA are shown in Fig. 8:3). The post-exercise period was accompanied by a rise in the level of ketone bodies (Fig. 8:4). In the control subjects HGH showed a sharp rise towards the end of the exercise but the subject with hypopituitarism showed no such increase (Table 8).

### Discussion

The results in the previous section suggest that a possible cause of the post-exercise ketosis seen in unfit subjects might be a relatively excessive HGH release and consequently a greater rise in FFA in these unfit subjects compared to the trained athletes. These results, however, indicate that exercise is accompanied by raised FFA and also by post-exercise ketosis even when there is no change in the HGH output. A rise in FFA after exercise in hypopituitary subjects has also been reported by Basu et al. (1960) and after starvation in hypophysectomized dogs by Goodman and Anobil (1961).

The ketotic response itself cannot stimulate the release of growth hormone because the timing of release is wrong and ingested acetoacetate caused no rise in plasma HGH levels (Chapter 7), although it was accompanied by a fall in blood glucose, a stimulus v

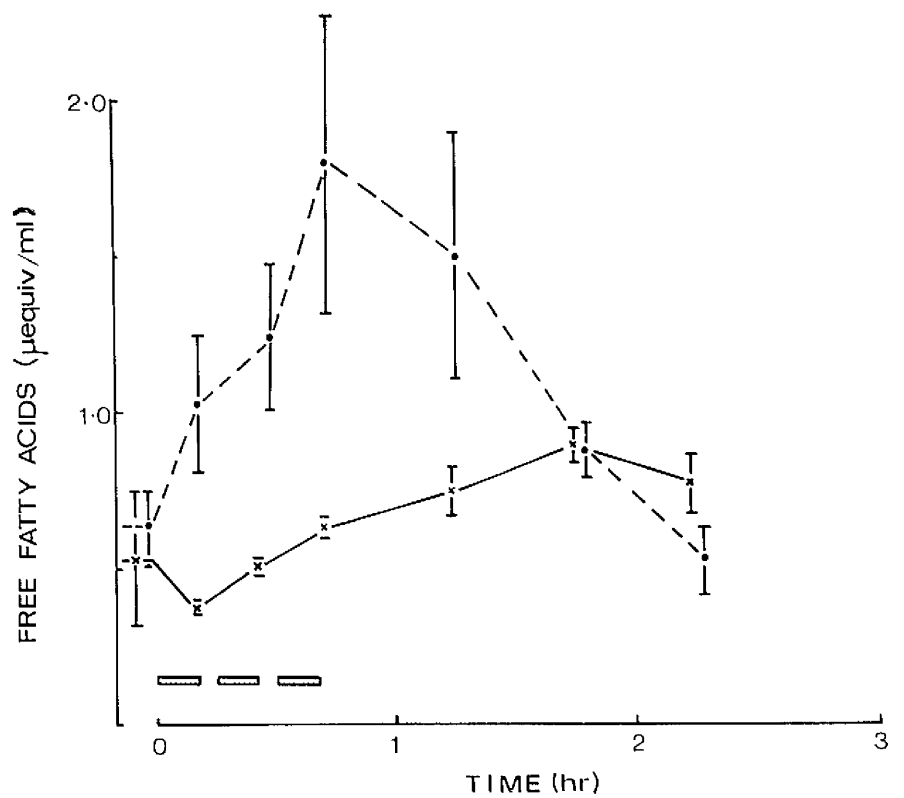


Fig. 8:3 Plasma free fatty acids (mean  $\pm$  1 S.D.) in 3 normal subjects (x) and 3 hypopituitary subjects (•) during and after exercise (stippled bars).

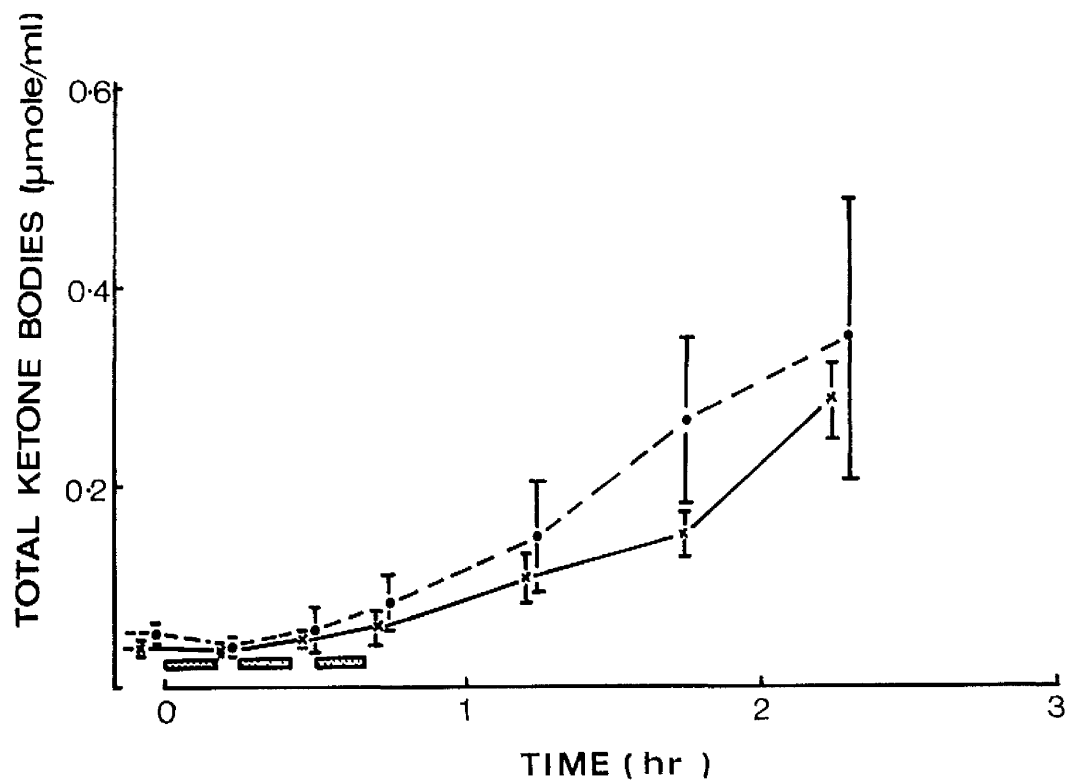


Fig. 8:4 Total blood ketone bodies (mean  $\pm$  1 S.D.) in 3 normal subjects (x) and in 3 hypopituitary subjects (•) during and after exercise (stippled bars).

	Rest	during exercise			min after end of exercise		
		after 1st 10 min	after 2nd 10 min	after 3rd 10 min	30 min	60 min	90 min
Controls 1	20.5	20.2	32.4	43.5	19.8	3.9	0
2	0	28.7	26.6	40.6	60.5	15.4	-
3	8.9	8.9	8.9	20.0	15.2	4.2	1.3
Subjects 1	1.3	0	1.4	0	0	0	0
2	-	0	0	0	0	-	-
3	6.7	6.2	6.7	6.3	3.8	7.4	7.0

Table 8:5 HGH (ng/ml) levels in 3 normal controls and in 3 hypopituitary subjects before, during and after three 10 min periods of exercise.

is known to cause HGH secretion when induced by insulin (Roth et al., 1963) or tolbutamide (Hunter and Greenwood, 1964). The rise in blood glycerol during exercise has been shown to occur before HGH rises (Hartog et al., 1967) so it is unlikely that lipolysis itself is acting as a stimulus for release.

It appears that differences in HGH release are not the sole explanation of different metabolic responses of trained and untrained subjects. Catecholamines also stimulate release of FFA and the possibility that athletes release less catecholamine or are more sensitive to these hormones requires study.

Original publications produced on this research

( to December 1969 )

1. Corbett, J.L., Johnson, R.H., Krebs, H.A.,  
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The effect of exercise on blood ketone body  
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## ABSTRACT

This thesis examines the effect of exercise on the blood concentrations of various metabolites with particular reference to differences between subjects who were athletically trained and others who were not in a state of athletic training.

In the first two chapters the biochemical techniques that have been used are described and normal values for the blood levels of acetoacetate and 3-hydroxybutyrate reported.

Chapter 3 describes the response of athletes and non-athletes to strenuous exercise ( $1\frac{1}{2}$  hours of running). The untrained subjects had more marked changes in lactate and pyruvate levels during the initial period of exercise than the trained subjects. After exercise the untrained subjects also had higher concentrations of plasma free fatty acids and total ketone bodies. Similar differences were obtained between fit and unfit subjects exercising for a short period ( $\frac{1}{2}$  hour) at similar work loads on a bicycle ergometer.

The development of post-exercise ketosis is negatively correlated with an individual's fitness as measured by the Harvard Step Test, but not with his percentage body fat, although ketone bodies are degradation products of fat metabolism (Chapter 4). The

degree of ketosis which develops depends on the duration of exercise (Chapter 5) and upon the amount of training that an individual has carried out; thus athletes who are out of training become ketotic after exercise.

Post-exercise ketosis in unfit subjects is diminished by the administration of glucose and the resulting hyperglycaemia is accompanied by decreases in free fatty acids and glycerol (Chapter 6). A similar pattern of metabolite changes was observed after administration of glucose to subjects undergoing a prolonged fast. These findings suggest that the increased concentration of free fatty acids results in a continued high rate of ketone body formation, which together with the decreased utilisation after exercise shows itself as post-exercise ketosis. The raised free fatty acid concentrations after exercise also cause inhibition of glucose utilisation resulting in a change in the shape of the glucose tolerance curves after exercise with a higher peak and a delayed fall.

The experimental production of ketosis by the oral administration of sodium acetoacetate is described in Chapter 7. This indicates that the resistance to ketosis seen in the fitter subjects is not due to differences in the rates of utilisation of ketone bodies. When acetoacetate is given during exercise there is an increase in tolerance indicating that



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during the exercise ketone bodies are being used as a fuel. This supports previous observations (Chapter showing that a second period of running is accompanied by a fall in the blood concentrations of ketones.

After exercise tolerance to acetoacetate is markedly reduced in untrained subjects but little changed in the trained subjects. These observations suggest that post-exercise ketosis in the untrained subjects develops because production exceeds utilisation.

In the last Chapter the effect of exercise on the secretion of growth hormone is compared in fit and unfit subjects. The latter are shown to have a more pronounced and long-lasting rise in secretion. The phenomenon however cannot wholly account for differences in fat metabolism between the two groups because subjects with hypopituitarism (and hence no increase in growth hormone levels with exercise) also developed post-exercise ketosis.

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